

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
6 September 2002 (06.09.2002)

PCT

(10) International Publication Number  
**WO 02/068588 A2**

(51) International Patent Classification<sup>7</sup>: C12N

(21) International Application Number: PCT/US02/04878

(22) International Filing Date: 20 February 2002 (20.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/269,876 21 February 2001 (21.02.2001) US

(71) Applicant (for all designated States except US): HUMAN  
GENOME SCIENCES, INC. [US/US]; 9410 Key West  
Avenue, Rockville, MD 20850 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): RUBEN, Steven, M.  
[US/US]; 18528 Heritage Hills Drive, Olney, MD 20832  
(US). ROSEN, Craig, A. [US/US]; 22400 Rolling Hill  
Lane, Laytonsville, MD 20882 (US). MOORE, Paul, A.  
[GB/US]; 19005 Leatherbark Drive, Germantown, MD  
20874 (US).

(74) Agents: MARTINEAU, Janet, M. et al.; Human Genome  
Sciences, Inc., 9410 Key West Avenue, Rockville, MD  
20850 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,  
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,  
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,  
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,  
VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),  
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,  
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent  
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,  
NE, SN, TD, TG).

**Published:**

— without international search report and to be republished  
upon receipt of that report

*For two-letter codes and other abbreviations, refer to the "Guidance  
Notes on Codes and Abbreviations" appearing at the beginning  
of each regular issue of the PCT Gazette.*

(54) Title: CYTOKINE RECEPTOR COMMON GAMMA CHAIN LIKE

(57) Abstract: The present invention relates to a novel human gene encoding a polypeptide which is a member of the Cytokine Receptor family. More specifically, the present invention relates to a polynucleotide encoding a novel human polypeptide named Cytokine Receptor Common Gamma Chain Like, or "CRGCL." This invention also relates to CRGCL polypeptides, as well as vectors, host cells, antibodies directed to CRGCL polypeptides, and the recombinant methods for producing the same. Also provided are diagnostic methods for detecting disorders related to the immune system, and therapeutic methods for treating, diagnosing, detecting, and/or preventing such disorders. The invention further relates to screening methods for identifying agonists and antagonists of CRGCL activity.

## Cytokine Receptor Common Gamma Chain Like

### *Field of the Invention*

[0001] The present invention relates to a novel human gene encoding a polypeptide which is a member of the Cytokine Receptor family. More specifically, the present invention relates to a polynucleotide encoding a novel human polypeptide named Cytokine Receptor Common Gamma Chain Like, or "CRCGCL." This invention also relates to CRCGCL polypeptides, as well as vectors, host cells, antibodies directed to CRCGCL polypeptides, and the recombinant methods for producing the same. Also provided are diagnostic methods for detecting disorders related to the immune system, and therapeutic methods for treating diagnosing, detecting, and/or preventing such disorders. The invention further relates to screening methods for identifying agonists and antagonists of CRCGCL activity.

### *Background of the Invention*

[0002] Biochemical and physiological effects often result from the binding of a cytokine to a specific receptor molecule. Receptor binding then stimulates certain, and often independent, signal transduction pathways. (Kishimoto, T., et al., Cell 76:253-262 (1994.) The interaction between a cytokine and a receptor is a primary regulator of a variety of cellular processes, including activation, proliferation, and differentiation. (Arai, K. -I, et al., Ann. Rev. Biochem. 59:783-836 (1990); Paul, W. E. and Seder, R. A., Cell 76:241-251 (1994)).

[0003] Of all the cytokine receptor signaling systems, IL-2 and its receptor complex (IL-2R) is one of the best studied. IL-2 is a cytokine that plays a pivotal role in the regulation of T cell-mediated immune responses. The IL-2R consists of three subunits: the IL-2 receptor alpha chain (IL-2R alpha); IL-2 receptor beta chain (IL-2R beta); and the common gamma chain (gamma chain). The gamma chain is also shared by other cytokine receptors.

[0004] Cytokines that bind to the interleukin-2 (IL-2) receptor common gamma chain (gamma c), including IL-2, IL-4, IL-7, IL-9, and IL-15, are important for the growth and differentiation of immune cells, such as T and B lymphocytes, natural killer cells, macrophages, and monocytes. These cytokines have overlapping biological effects that in part result from the use of the shared receptor subunit gamma c. Recently it has been shown

that these cytokines activate a number of important intracellular signaling molecules, including the Janus kinases JAK1 and JAK3 and members of the transcription factor family of signal transducers and activators of transcription (STATs), by binding to the interleukin-2 (IL-2) receptor complex.

[0005] The discovery of these signaling pathways has led to important new insights into their role in lymphocyte maturation, as it has emerged that mutations in the genes encoding both gamma c and JAK3 result in similar forms of severe combined immunodeficiency (SCID). For example, mutations in the human interleukin-2 (IL-2) receptor gamma, mapped to the X chromosome, is associated with X-linked severe combined immunodeficiency. (Human Molecular Genetics, 2(8): 1099 (1993).)

[0006] Thus, there is a need for polypeptides that regulate the differentiation and proliferation of T and/or B cells, since disturbances of such regulation may be involved in disorders relating to immune system. Therefore, there is a need for identification and characterization of such human polypeptides which can play a role in detecting, preventing, ameliorating or correcting such disorders.

### *Summary of the Invention*

[0007] The present invention relates to a novel polynucleotide and the encoded polypeptide of CRCGCL. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant or synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting and/or diagnosing disorders related to the polypeptides, and therapeutic methods for treating, detecting, diagnosing and/or preventing such disorders. The invention further relates to screening methods for identifying binding partners of CRCGCL.

[0008] In accordance with one embodiment of the present invention, there is provided a novel mature CRCGCL polypeptide, as well as biologically active and diagnostically or therapeutically useful fragments, analogs and derivatives thereof.

[0009] In accordance with another embodiment of the present invention, there are provided isolated nucleic acid molecules encoding human CRCGCL, including mRNAs, DNAs, cDNAs, genomic DNAs as well as analogs and biologically active and diagnostically or therapeutically useful fragments and derivatives thereof.

[0010] This cytokine receptor is named CRGCL and the invention includes CRGCL polypeptides having at least a portion of the amino acid sequence in Figures 1A-B (SEQ ID NO:2) or amino acid sequence encoded by the cDNA clone (HTAEK53) which was deposited on February 25, 1998, assigned ATCC number 209641, and deposited again on March 23, 1998, assigned ATCC Deposit number 209691. The nucleotide sequence determined by sequencing the deposited CRGCL clone, which is shown in Figures 1A-B (SEQ ID NO:1), contains an open reading frame encoding a complete polypeptide of 371 amino acid residues including an N-terminal methionine (i.e., amino acid residues 1-371 of SEQ ID NO:2), a predicted signal peptide of about 22 amino acid residues (i.e., amino acid residues 1-22 of SEQ ID NO:2), a predicted mature form of about 349 amino acids (i.e., amino acid residues 23-371 of SEQ ID NO:2), and a deduced molecular weight for the complete protein of about 37 kDa.

[0011] Thus, one embodiment of the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a polypeptide comprising, or alternatively consisting of, the full-length CRGCL polypeptide (amino acid residues 1 to 371 in Figures 1A-B (SEQ ID NO:2) or as encoded by the cDNA clone contained in the deposit having ATCC accession number 209691 or 209641; (b) a nucleotide sequence encoding a polypeptide comprising or alternatively consisting of, the CRGCL extracellular domain (predicted to constitute amino acid residues from about 23 to about 225 in Figures 1A-B (SEQ ID NO:2) or as encoded by the clone contained in the deposit having ATCC accession number 209691 or 209641; (c) a nucleotide sequence encoding a fragment of the polypeptide of (b) having CRGCL functional activity (e.g., biological activity, such as the ability to promote B cell lymphopoiesis; ability to costimulate thymocytes or mature T cells, ability to promote the proliferation, differentiation, and/or chemotaxis of T cells; ability to promote the release of T-cell attracting chemokines from monocytes; ability to enhance the T cell stimulatory capacity of CD11c+ subset of dendritic cells; and/or ability to activate STAT5B; antigenicity, such as the ability to bind (or compete with a CRGCL polypeptide for binding) to an anti-CRGCL antibody; immunogenicity, such as the ability to generate an antibody which binds to a CRGCL polypeptide; ability to form multimers with CRGCL polypeptides of the invention; ability to form multimers with other type I cytokine receptor chains (e.g., IL-7 alpha chain); and/or ability to bind to a ligand for a CRGCL polypeptide (e.g., TSLP); (d) a nucleotide sequence encoding a polypeptide comprising the CRGCL



intracellular domain (predicted to constitute amino acid residues from about 261 to about 371 in Figures 1A-B (SEQ ID NO:2)) or as encoded by the clone contained in the deposit having ATCC accession number 209623; (e) a nucleotide sequence encoding a polypeptide comprising, or alternatively consisting of, the CRCGCL transmembrane domain (predicted to constitute amino acid residues from about 226 to about 260 in Figures 1A-B (SEQ ID NO:2)) or as encoded by the cDNA clone contained in the deposit having ATCC accession number 209691 or 209641; (f) a nucleotide sequence encoding a polypeptide comprising, or alternatively consisting of, a soluble CRCGCL polypeptide having the extracellular and intracellular domains but lacking the transmembrane domain; and (g) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e) or (f) above.

[0012] Further embodiments of the invention include isolated nucleic acid molecules that comprise, or alternatively consist of, a polynucleotide having a nucleotide sequence at least 80%, 85% or 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g) above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a), (b), (c), (d), (e), (f) or (g) above. This polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

[0013] In additional embodiments, the nucleic acid molecules of the invention comprise, or alternatively consist of, a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a CRCGCL polypeptide having an amino acid sequence in (a), (b), (c), (d), (e), (f) or (g) above. A further nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide which encodes the amino acid sequence of a CRCGCL polypeptide having an amino acid sequence which contains at least one amino acid addition, substitution, and/or deletion but not more than 50 amino acid additions, substitutions and/or deletions, even more preferably, not more than 40 amino acid additions, substitutions, and/or deletions, still more preferably, not more than 30 amino acid additions, substitutions, and/or deletions, and still even more preferably, not more than 20 amino acid additions, substitutions, and/or deletions. Of course, in order of ever-increasing preference, it is highly preferable for a polynucleotide which encodes the amino acid sequence of a CRCGCL polypeptide to have an amino acid sequence which contains not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 or 1-100, 1-50, 1-25, 1-20, 1-15, 1-

10, or 1-5 amino acid additions, substitutions and/or deletions. Conservative substitutions are preferable.

[0014] The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of CRCGCL polypeptides by recombinant techniques.

[0015] In accordance with a further embodiment of the present invention, there is provided a process for producing such polypeptides by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing a CRCGCL nucleic acid sequence of the invention, under conditions promoting expression of said polypeptide and subsequent recovery of said polypeptide.

[0016] The invention further provides an isolated CRCGCL polypeptide comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of: (a) the amino acid sequence of the full-length CRCGCL polypeptide having the complete amino acid sequence shown in Figures 1A-B (i.e., positions 1-371 of SEQ ID NO:2) or as encoded by the cDNA plasmid contained in the deposit having ATCC accession number 209691 or 209641; (b) the amino acid sequence of the full-length CRCGCL polypeptide having the complete amino acid sequence shown in SEQ ID NO:2 excepting the N-terminal methionine (i.e., positions 2 to 371 of SEQ ID NO:2); (c) a fragment of the polypeptide of (b) having CRCGCL functional activity (e.g., biological activity, such as the ability to promote B cell lymphopoiesis; ability to costimulate thymocytes or mature T cells, ability to promote the proliferation, differentiation, and/or chemotaxis of T cells; ability to promote the release of T-cell attracting chemokines from monocytes; ability to enhance the T cell stimulatory capacity of CD11c<sup>+</sup> subset of dendritic cells; and/or ability to activate STAT5B; antigenicity, such as the ability to bind (or compete with a CRCGCL polypeptide for binding) to an anti-CRCGCL antibody; immunogenicity, such as the ability to generate an antibody which binds to a CRCGCL polypeptide; ability to form multimers with CRCGCL polypeptides of the invention; ability to form multimers with other type I cytokine receptor chains (e.g., IL-7 alpha chain); and/or ability to bind to a ligand for a CRCGCL polypeptide (e.g., TSLP); (d) the amino acid sequence of the predicted extracellular domain of the CRCGCL polypeptide having the amino acid sequence at positions 23 to 225 in Figures 1A-B (SEQ ID NO:2) or as encoded by the cDNA plasmid contained in the deposit having ATCC accession number 209691 or 209641; (e) the amino acid sequence of the CRCGCL intracellular domain

(predicted to constitute amino acid residues from about 261 to about 371 in Figures 1A-B (SEQ ID NO:2)) or as encoded by the cDNA plasmid contained in the deposit having ATCC accession number 209691 or 209641; (f) the amino acid sequence of the CRCGCL transmembrane domain (predicted to constitute amino acid residues from about 226 to about 260 in Figures 1A-B (SEQ ID NO:2)) or as encoded by the cDNA plasmid contained in the deposit having ATCC accession number 209691 or 209641; (g) the amino acid sequence of the soluble CRCGCL polypeptide having the extracellular and intracellular domains but lacking the transmembrane domain, wherein each of these domains is defined above; and (h) fragments of the polypeptide of (a), (b), (c), (d), (e), (f) or (g). The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 85% or 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described in (a), (b), (c), (d), (e), (f) or (g) above, as well as polypeptides having an amino acid sequence with at least 80%, 85%, or 90% similarity, and more preferably at least 95% similarity, to those above. Additional embodiments of the invention relates to polypeptides which comprise, or alternatively consist of, the amino acid sequence of an epitope-bearing portion of a CRCGCL polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e), (f), (g) or (h) above. Polypeptides having the amino acid sequence of an epitope-bearing portion of a CRCGCL polypeptide of the invention include portions of such polypeptides with at least 4, at least 5, at least 6, at least 7, at least 8, and preferably at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and more preferably at least about 30 amino acids to about 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the invention described above also are included in the invention.

[0017] The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these polynucleotides and nucleic acid molecules are also encompassed by the invention.

[0018] Certain non-exclusive embodiments of the invention relate to a polypeptide which has the amino acid sequence of an epitope-bearing portion of a CRCGCL polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e), (f), (g), (h) or (i) above. In other embodiments, the invention provides an isolated antibody that binds specifically (i.e., uniquely) to a CRCGCL polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e), (f), (g), (h) or (i) above.

[0019] The invention further provides methods for isolating antibodies that bind specifically (i.e., uniquely) to a CRCGCL polypeptide having an amino acid sequence as described herein. Such antibodies may be agonistic or antagonistic and are useful diagnostically or therapeutically as described below.

[0020] The invention also provides for pharmaceutical compositions comprising soluble CRCGCL polypeptides, particularly human CRCGCL polypeptides, and/or anti-CRCGCL antibodies which may be employed, for instance, to treat, prevent, prognose and/or diagnose tumor and tumor metastasis, infections by bacteria, viruses and other parasites, immunodeficiencies, inflammatory diseases, lymphadenopathy, autoimmune diseases, graft versus host disease, stimulate peripheral tolerance, destroy some transformed cell lines, mediate cell activation, survival and proliferation, to mediate immune regulation and inflammatory responses, and to enhance or inhibit immune responses.

[0021] In certain embodiments, soluble CRCGCL polypeptides of the invention, agonists and/or antagonist thereof, are administered, to treat, prevent, prognose and/or diagnose an immunodeficiency (e.g., severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVID) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency) or conditions associated with an immunodeficiency.

[0022] In a specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, prognose and/or diagnose common variable immunodeficiency.

[0023] In a specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, prognose and/or diagnose X-linked agammaglobulinemia.

[0024] In another specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, prognose and/or diagnose severe combined immunodeficiency (SCID).

[0025] In another specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, prognose and/or diagnose Wiskott-Aldrich syndrome.

[0026] In another specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, prognose and/or diagnose X-linked Ig deficiency with hyper IgM.

[0027] In another embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof are administered to treat, prevent, prognose and/or diagnose an autoimmune disease (e.g., rheumatoid arthritis, systemic lupus erythematosus, idiopathic thrombocytopenia purpura, autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, autoimmune hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g. IgA nephropathy), Multiple Sclerosis, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henloch-Schoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis, Goodpasture's syndrome, Pemphigus, Receptor autoimmunities such as, for example, (a) Graves' Disease, (b) Myasthenia Gravis, and (c) insulin resistance, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, scleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis/dermatomyositis, pernicious anemia, idiopathic Addison's disease, infertility, glomerulonephritis such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid, Sjogren's syndrome, diabetes mellitus, and adrenergic drug resistance (including adrenergic drug resistance with asthma or

cystic fibrosis), chronic active hepatitis, primary biliary cirrhosis, other endocrine gland failure, vitiligo, vasculitis, post-MI, cardiomy syndrome, urticaria, atopic dermatitis, asthma, inflammatory myopathies, and other inflammatory, granulomatous, degenerative, and atrophic disorders) or conditions associated with an autoimmune disease. In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, prognosed and/or diagnosed using CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof. In another specific preferred embodiment, systemic lupus erythematosus is treated, prevented, prognosed, and/or diagnosed using CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof. In another specific preferred embodiment, idiopathic thrombocytopenia purpura is treated, prevented, prognosed, and/or diagnosed using CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof. In another specific preferred embodiment IgA nephropathy is treated, prevented, prognosed and/or diagnosed using CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof. In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, prognosed and/or diagnosed using CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof.

[0028] The invention further provides compositions comprising a CRCGCL polynucleotide, a CRCGCL polypeptide, and/or an anti-CRCGCL antibody, for administration to cells *in vitro*, to cells *ex vivo*, and to cells *in vivo*, or to a multicellular organism. In preferred embodiments, the compositions of the invention comprise a CRCGCL polynucleotide for expression of a CRCGCL polypeptide in a host organism for treatment of disease. In a most preferred embodiment, the compositions of the invention comprise a CRCGCL polynucleotide for expression of a CRCGCL polypeptide in a host organism for treatment of an immunodeficiency and/or conditions associated with an immunodeficiency. Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant endogenous activity of a CRCGCL gene.

[0029] The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a cellular response induced by CRCGCL which involves contacting cells which express CRCGCL with the candidate compound, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made in absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the

compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

[0030] In another embodiment, a method for identifying CRCGCL ligands is provided, as well as a screening assay for agonists and antagonists directed to such ligands. This assay involves determining the effect a candidate compound has on CRCGCL binding to the CRCGCL ligand (e.g., TSLP). In particular, the method involves contacting a CRCGCL ligand (e.g., TSLP) with a CRCGCL polypeptide of the invention and a candidate compound and determining whether CRCGCL polypeptide binding to the CRCGCL ligand (e.g., TSLP) is increased or decreased due to the presence of the candidate compound. The antagonists may be employed to prevent septic shock, inflammation, cerebral malaria, activation of the HIV virus, graft-host rejection, bone resorption, rheumatoid arthritis, cachexia (wasting or malnutrition), immune system function, lymphoma, and autoimmune disorders (e.g., rheumatoid arthritis and systemic lupus erythematosus).

[0031] The present invention further encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to a CRCGCL polypeptide or polypeptide fragment or variant of CRCGCL. In particular, the invention encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to a polypeptide or polypeptide fragment or variant of human CRCGCL such as those of SEQ ID NO:2.

[0032] The present invention relates to methods and compositions for preventing, treating or ameliorating a disease or disorder comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to CRCGCL or a fragment or variant thereof. In specific embodiments, the present invention relates to methods and compositions for preventing, treating or ameliorating a disease or disorder associated with CRCGCL function or CRCGCL ligand function or aberrant CRCGCL or CRCGCL ligand expression, comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to CRCGCL or a fragment or variant thereof.

[0033] The present invention also encompasses methods and compositions for detecting, diagnosing, or prognosing diseases or disorders comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants

thereof, or related molecules, that immunospecifically bind to CRCGCL or a fragment or variant thereof. In specific embodiments, the present invention also encompasses methods and compositions for detecting, diagnosing, or prognosing diseases or disorders associated with CRCGCL function or CRCGCL ligand function or aberrant CRCGCL or CRCGCL ligand expression, comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to CRCGCL or a fragment or variant thereof.

[0034] Another embodiment of the present invention includes the use of the antibodies of the invention as a diagnostic tool to monitor the expression of CRCGCL expression on cells.

[0035] The present invention also provides antibodies that bind one or more CRCGCL polypeptides that act as either CRCGCL agonists or CRCGCL antagonists.

### *Brief Description of the Drawings*

[0036] Figures 1A-1B show the nucleotide sequence (SEQ ID NO:1) and the deduced amino acid sequence (SEQ ID NO:2) of CRCGCL. The predicted leader sequence is located at about amino acids 1-22.

[0037] Figure 2 shows the regions of identity between the amino acid sequence of the CRCGCL protein and the translation product of the closest homolog, the Bos Taurus Interleukin-2 receptor gamma (Accession Nos. 1532088) (SEQ ID NO:3), determined by BLAST analysis. Identical amino acids between the two polypeptides are shaded in black, while conservative amino acids are boxed. By examining the regions of amino acids shaded and/or boxed, the skilled artisan can readily identify conserved domains between the two polypeptides. These conserved domains are preferred embodiments of the present invention.

[0038] Figure 3 shows an analysis of the CRCGCL amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, and all were generated using the default settings. In the "Antigenic Index or Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the CRCGCL protein, i.e., regions from which epitope-bearing peptides of the invention can be obtained. The domains defined by these



graphs are contemplated by the present invention. Tabular representation of the data summarized graphically in Figure 3 can be found in Table 1. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIV. The column headings refer to the following features of the amino acid sequence presented in Figure 3, and Table 1: "Res": amino acid residue of SEQ ID NO:2 and Figures 1A and 1B; "Position": position of the corresponding residue within SEQ ID NO:2 and Figures 1A and 1B; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface Probability Plot - Emini.

### *Detailed Description*

#### Definitions

[0039] The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

[0040] In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. However, a nucleic acid contained in a clone that is a member of a library (e.g., a genomic or cDNA library) that has not been isolated from other members of the library (e.g., in the form of a homogeneous solution containing the clone and other members of the library) or a chromosome removed from a cell or a cell lysate (e.g., a "chromosome spread", as in a karyotype), or a preparation

of randomly sheared genomic DNA or a preparation of genomic DNA cut with one or more restriction enzymes is not "isolated" for the purposes of this invention. As discussed further herein, isolated nucleic acid molecules according to the present invention may be produced naturally, recombinantly, or synthetically.

[0041] In the present invention, a "secreted" CRCGCL protein refers to a protein capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as a CRCGCL protein released into the extracellular space without necessarily containing a signal sequence. If the CRCGCL secreted protein is released into the extracellular space, the CRCGCL secreted protein can undergo extracellular processing to produce a "mature" CRCGCL protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

[0042] As used herein, a CRCGCL "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:1 or the cDNA contained within the clone deposited with the ATCC. For example, the CRCGCL polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a CRCGCL "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined. However, one embodiment of the present invention does not include the polynucleotide sequence of Genbank Accession No. X91553, herein incorporated by reference.

[0043] In specific embodiments, the polynucleotides of the invention are less than 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, or 7.5 kb in length. In a further embodiment, polynucleotides of the invention comprise at least 15 contiguous nucleotides of CRCGCL coding sequence, but do not comprise all or a portion of any CRCGCL intron. In another embodiment, the nucleic acid comprising CRCGCL coding sequence does not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the CRCGCL gene in the genome).

[0044] In the present invention, the full length CRCGCL sequence identified as SEQ ID NO:1 was generated by overlapping sequences of the deposited clone (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:1 was deposited with the American Type Culture Collection ("ATCC") on February 25, 1998, and given ATCC Deposit Number 209641. A second clone was also deposited with the ATCC on March 23, 1998, and was given the ATCC Deposit Number 209691. The ATCC is located at

10801 University Boulevard, Manassas, VA 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

[0045] A CRCGCL "polynucleotide" also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:1, the complement thereof, or the cDNA within the deposited clone. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

[0046] Also contemplated are nucleic acid molecules that hybridize to the CRCGCL polynucleotides at moderately high stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, moderately high stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

[0047] Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

[0048] Of course, a polynucleotide which hybridizes only to polyA<sup>+</sup> sequences (such as any 3' terminal polyA<sup>+</sup> tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

[0049] The CRCGCL polynucleotide can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, CRCGCL polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the CRCGCL polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. CRCGCL polynucleotides may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

[0050] CRCGCL polypeptides can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The CRCGCL polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the CRCGCL polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given CRCGCL polypeptide. Also, a given CRCGCL polypeptide may contain many types of modifications. CRCGCL polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic CRCGCL polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, biotinylation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, derivatization by known protecting/blocking groups, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of

pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, linkage to an antibody molecule or other cellular ligand, methylation, myristoylation, oxidation, pegylation, proteolytic processing (e.g., cleavage), phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

[0051] "SEQ ID NO:1" refers to a CRCGCL polynucleotide sequence while "SEQ ID NO:2" refers to a CRCGCL polypeptide sequence.

[0052] A CRCGCL polypeptide "having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a CRCGCL polypeptide, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the CRCGCL polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the CRCGCL polypeptide (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the CRCGCL polypeptide.)

#### **CRCGCL Polynucleotides and Polypeptides**

[0053] Clone HTAEK53 was isolated from an activated T-cell cDNA library. Initially, the sequence of clone HTAEK53 was identified as SEQ IDNO:26 and the deduced amino acid sequence was predicted as SEQ ID NO:27, with a recognition that an apparent frame shift in the sequence existed. This frame shift was easily resolved using standard molecular biology techniques, generating the nucleotide sequence of SEQ ID NO:1 and the deduced amino acid sequence shown in SEQ ID NO:2.

[0054] The deposited clone contains a cDNA having a total of 1573 nucleotides, which encodes a predicted open reading frame of 371 amino acid residues. (See Figures 1A-1B.) The open reading frame begins at a N-terminal methionine located at nucleotide position 13,

and ends at a stop codon at nucleotide position 1128. The predicted molecular weight of the CRCGCL protein should be about 42 kDa.

[0055] Subsequent Northern analysis also showed a 1.6 Kb transcript in a cervical cancer cell line (HeLa), activated T cells, and a lung carcinoma cell line (A549), while a shorter variant is also expressed in the lymph node and to a lesser extent in the spleen tissues, a pattern consistent with immune specific expression.

[0056] CRCGCL expression was not observed in the following cell lines, HL60, K562, Molt-4, Raji, SW480, G361, as well as the heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, thymus, prostate, testis, ovary, small intestine, colon, or peripheral blood leukocytes, a pattern consistent with immune specific expression.

[0057] Using BLAST analysis, SEQ ID NO:2 was found to be homologous to members of the Cytokine Receptor family. Particularly, SEQ ID NO:2 contains domains homologous to the translation product of the Bos Taurus mRNA for Interleukin-2 receptor gamma (Accession Nos. 1532088) (Figure 2) (SEQ ID NO:3), including the following conserved domains: (a) a predicted transmembrane domain located at about amino acids 226-260; (b) a predicted WXWS (SEQ ID NO:30) or [STGL]-x-W-[SG]-x-W-S (SEQ ID NO:18) domain located at about amino acids 198-204 (T-x-P-S-x-W-S) (SEQ ID NO:19), although not a perfect match; and (c) a predicted Jak Box, having the motif W(P,E)X(V,I)P(N,S,D)P (SEQ ID NO:20) domain located at about amino acids 261-268 (I-P-X-V-P-D-P) (SEQ ID NO:21), although not a perfect match. These polypeptide fragments of CRCGCL are specifically contemplated in the present invention. Because Interleukin-2 receptor gamma (Accession Nos. 1532088) is thought to be important as a cytokine receptor, the homology between Interleukin-2 receptor gamma (Accession Nos. 1532088) and CRCGCL suggests that CRCGCL may also be involved in the differentiation and proliferation of cells. CRCGCL is also homologous to other Interleukin-2 receptor gamma genes isolated from a variety of species, such as human (Accession No. gi/349632), Canis familiaris (Accession No. gi/517412), and mouse (pri/S37582).

[0058] Moreover, the encoded polypeptide has a predicted leader sequence located at about amino acids 1-22. (See Figures 1A-1B.) Also shown in Figures 1A-1B, one embodiment of the secreted form of CRCGCL encompasses about amino acids 23-371, amino acids 23-225, or amino acids 1-231. These polypeptide fragments of CRCGCL are specifically contemplated in the present invention.

[0059] Further, specifically contemplated is the soluble form of CRCGCL. Particularly preferred embodiments of the invention encompass the use of the soluble form of CRCGCL as an antagonist of CRCGCL, such as, for example, to inhibit the proliferation, differentiation and/or chemotaxis of T cells.

[0060] Other preferred polypeptide fragments comprise the amino acids sequence: QIQIIFYNLETVQVTWNASKYSRTNLTFHYRFNGDEAYDQCTNYLLQEGHTSGC (SEQ ID NO:22); RRHSLFLHQEWDAPRFHRKSLDGLLPETQF (SEQ ID NO:23); LLYEVQYRSPFDTEWQSKQENTCNVTIEGLDAEKCYSFVVRVKAMEDVYGPDTYPS DWSEVTCWQRGEIRDACAETPTPPK (SEQ ID NO:24); and/or MEDVYGPDTYPSDWSEVTCWQRGEIRDACAETPTPPKPKLSKFILISSLAILLMVSL LLSLWKLWRXKKFLXPSVPDPKSIFPGLFXIHQGNFQEWITDTQNV AHLHKMAGAE QESGPPEPLVVQLAKTEAESPRMLDPQTEEKEASGGSLLQLPHQPLQGGDVVTIGGFT FVMNDRSYVA (SEQ ID NO:25), as well as fragments thereof. Also preferred are polynucleotide fragments encoding these polypeptide fragments.

[0061] Because CRCGCL was isolated from activated T cells, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of immune disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the immune system, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

[0062] The tissue distribution in only activated T-cells and homology to the cytokine receptors IL2 and IL13 suggests that this protein is a novel member of the cytokine receptor family expressed in immune tissues, specifically on T-cells, and may be upregulated in other lympho-hematopoietic cells. The tissue distribution of this gene in cells of the immune system suggests that the protein product of this clone would be useful for treatment, prophylaxis, detection and/or diagnosis of immune and autoimmune diseases, such as lupus, transplant rejection, allergic reactions, arthritis, asthma, immunodeficiency diseases, leukemia, AIDS. In addition its expression in T-cells suggests a potential role in the treatment, prophylaxis, diagnosis and/or detection of thymus disorders such as Graves

Disease, lymphocytic thyroiditis, hyperthyroidism and hypothyroidism. The receptor could also serve as a target for small molecule or monoclonal antibody, blocking its activity, which could be important in the disease states listed herein.

[0063] The CRCGCL nucleotide sequence identified as SEQ ID NO:1 was assembled from partially homologous ("overlapping") sequences obtained from the deposited clone, and in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:1.

[0064] Therefore, SEQ ID NO:1 and the translated SEQ ID NO:2 are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:1 is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:1 or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:2 may be used to generate antibodies which bind specifically to CRCGCL.

[0065] Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

[0066] Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:1 and the predicted translated amino acid sequence identified as SEQ ID NO:2, but also a sample of plasmid DNA containing a human cDNA of CRCGCL deposited with the ATCC. The nucleotide sequence of the deposited CRCGCL clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted CRCGCL amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by the deposited clone



can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human CRCGCL cDNA, collecting the protein, and determining its sequence.

[0067] The present invention also relates to the CRCGCL gene corresponding to SEQ ID NO:1, SEQ ID NO:2, or the deposited clone. The CRCGCL gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the CRCGCL gene from appropriate sources of genomic material.

[0068] Also provided in the present invention are species homologs of CRCGCL. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for the desired homologue.

[0069] The CRCGCL polypeptides can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

[0070] The CRCGCL polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

[0071] CRCGCL polypeptides are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a CRCGCL polypeptide, including the secreted polypeptide, can be substantially purified by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). CRCGCL polypeptides also can be purified from natural or recombinant sources using antibodies of the invention raised against the CRCGCL protein in methods which are well known in the art.

#### **Polynucleotide and Polypeptide Variants**

[0072] "Variant" refers to a polynucleotide or polypeptide differing from the CRCGCL polynucleotide or polypeptide, but retaining essential properties thereof. Generally, variants

are overall closely similar, and, in many regions, identical to the CRCGCL polynucleotide or polypeptide.

[0073] By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the CRCGCL polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown of SEQ ID NO:1, the ORF (open reading frame), or any fragment specified as described herein.

[0074] As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245.) In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

[0075] If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are

not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

[0076] For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

[0077] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0078] As a practical matter, whether any particular polypeptide is at least 80%, 85%,

90%, 92%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequences shown in SEQ ID NO:2 or to the amino acid sequence encoded by deposited DNA clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

[0079] If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

[0080] For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues

represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

[0081] The CRCGCL variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted; or added in any combination are also preferred. CRCGCL polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

[0082] Naturally occurring CRCGCL variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

[0083] Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the CRCGCL polypeptides. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., *J. Biol. Chem.* 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting

8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

[0084] Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

[0085] Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

[0086] Thus, the invention further includes CRCGCL polypeptide variants which show substantial functional activity (e.g., biological activity [ability to bind a CRCGCL ligand (e.g., TSLP); ability to promote B cell lymphopoiesis; ability to costimulate thymocytes or mature T cells; ability to promote the proliferation, differentiation, and/or chemotaxis of T cells; ability to promote the release of T-cell attracting chemokines from monocytes; ability to enhance the T cell stimulatory capacity of CD11c+ subset of dendritic cells; ability to complex with type I cytokine receptor chains, including but not limited to, IL-7R alpha chain; ability to activate the Jax/STAT pathway (e.g., by phosphorylation of, for example, STAT5B); ability to bind a CRCGCL specific antibody])). Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity.

[0087] The present application is directed to nucleic acid molecules at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences disclosed

herein, (e.g., encoding a polypeptide having the amino acid sequence of an N and/or C terminal deletion disclosed below as m-n of SEQ ID NO:2), irrespective of whether they encode a polypeptide having CRCGCL functional activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having CRCGCL functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having CRCGCL functional activity include, inter alia, (1) isolating a CRCGCL gene or allelic or splice variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the CRCGCL gene, as described in Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting CRCGCL mRNA expression in specific tissues.

[0088] Preferred, however, are nucleic acid molecules having sequences at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences disclosed herein, which do, in fact, encode a polypeptide having CRCGCL functional activity. By "a polypeptide having CRCGCL functional activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to a functional activity of the CRCGCL polypeptides of the present invention (e.g., complete (full-length) CRCGCL, mature CRCGCL and soluble CRCGCL (e.g., having sequences contained in the extracellular domain of CRCGCL) as measured, for example, in a particular immunoassay or biological assay. For example, a CRCGCL functional activity can routinely be measured by determining the ability of a CRCGCL polypeptide to bind a CRCGCL ligand (e.g., TSLP). CRCGCL functional activity may also be measured by determining the ability of a polypeptide, such as cognate ligand which is free or expressed on a cell surface, to induce cells expressing the polypeptide. In addition, CRCGCL functional activity can routinely be measured by determining the ability of a CRCGCL polypeptide to promote B cell lymphopoiesis, and/or costimulate thymocytes or mature T cells; ability to promote the proliferation, differentiation, and/or chemotaxis of T cells; ability to promote the release of T-cell attracting chemokines from monocytes; ability to enhance the T cell stimulatory capacity of CD11c<sup>+</sup> subset of dendritic cells; ability to generate a CRCGCL specific antibody; ability to activate of STAT5B; ability to bind (or compete with a CRCGCL polypeptide for binding) to an anti-CRCGCL antibody; ability to form multimers with CRCGCL polypeptides of the

invention; and/or ability to form multimers with other type I cytokine receptor chains (e.g., IL-7R alpha chain).

[0089] Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence of the deposited cDNA, the nucleic acid sequence shown in Figures 1A-1B (SEQ ID NO:1), or fragments thereof, will encode polypeptides "having CRCGCL functional activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having CRCGCL functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

[0090] For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

[0091] The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

[0092] The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.



[0093] As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

[0094] For example, site directed changes at the amino acid level of CRCGCL can be made by replacing a particular amino acid with a conservative amino acid. Preferred conservative mutations include: M1 replaced with A, G, I, L, S, T, or V; G2 replaced with A, I, L, S, T, M, or V; R3 replaced with H, or K; L4 replaced with A, G, I, S, T, M, or V; V5 replaced with A, G, I, L, S, T, or M; L6 replaced with A, G, I, S, T, M, or V; L7 replaced with A, G, I, S, T, M, or V; W8 replaced with F, or Y; G9 replaced with A, I, L, S, T, M, or V; A10 replaced with G, I, L, S, T, M, or V; A11 replaced with G, I, L, S, T, M, or V; V12 replaced with A, G, I, L, S, T, or M; F13 replaced with W, or Y; L14 replaced with A, G, I, S, T, M, or V; L15 replaced with A, G, I, S, T, M, or V; G16 replaced with A, I, L, S, T, M, or V; G17 replaced with A, I, L, S, T, M, or V; W18 replaced with F, or Y; M19 replaced with A, G, I, L, S, T, or V; A20 replaced with G, I, L, S, T, M, or V; L21 replaced with A, G, I, S, T, M, or V; G22 replaced with A, I, L, S, T, M, or V; Q23 replaced with N; G24 replaced with A, I, L, S, T, M, or V; G25 replaced with A, I, L, S, T, M, or V; A26 replaced with G, I, L, S, T, M, or V; A27 replaced with G, I, L, S, T, M, or V; E28 replaced with D; G29 replaced with A, I, L, S, T, M, or V; V30 replaced with A, G, I, L, S, T, or M; Q31 replaced with N; I32 replaced with A, G, L, S, T, M, or V; Q33 replaced with N; I34 replaced with A, G, L, S, T, M, or V; I35 replaced with A, G, L, S, T, M, or V; Y36 replaced with F, or W; F37 replaced with W, or Y; N38 replaced with Q; L39 replaced with A, G, I, S, T, M, or V; E40 replaced with D; T41 replaced with A, G, I, L, S, M, or V; V42 replaced with A, G, I, L, S, T, or M; Q43 replaced with N; V44 replaced with A, G, I, L, S, T, or M; T45 replaced with A, G, I, L, S, M, or V; W46 replaced with F, or Y; N47 replaced with Q; A48 replaced with G, I, L, S, T, M, or V; S49 replaced with A, G, I, L, T, M, or V; K50 replaced with H, or R;

Y51 replaced with F, or W; S52 replaced with A, G, I, L, T, M, or V; R53 replaced with H, or K; T54 replaced with A, G, I, L, S, M, or V; N55 replaced with Q; L56 replaced with A, G, I, S, T, M, or V; T57 replaced with A, G, I, L, S, M, or V; F58 replaced with W, or Y; H59 replaced with K, or R; Y60 replaced with F, or W; R61 replaced with H, or K; F62 replaced with W, or Y; N63 replaced with Q; G64 replaced with A, I, L, S, T, M, or V; D65 replaced with E; E66 replaced with D; A67 replaced with G, I, L, S, T, M, or V; Y68 replaced with F, or W; D69 replaced with E; Q70 replaced with N; T72 replaced with A, G, I, L, S, M, or V; N73 replaced with Q; Y74 replaced with F, or W; L75 replaced with A, G, I, S, T, M, or V; L76 replaced with A, G, I, S, T, M, or V; Q77 replaced with N; E78 replaced with D; G79 replaced with A, I, L, S, T, M, or V; H80 replaced with K, or R; T81 replaced with A, G, I, L, S, M, or V; S82 replaced with A, G, I, L, T, M, or V; G83 replaced with A, I, L, S, T, M, or V; L85 replaced with A, G, I, S, T, M, or V; L86 replaced with A, G, I, S, T, M, or V; D87 replaced with E; A88 replaced with G, I, L, S, T, M, or V; E89 replaced with D; Q90 replaced with N; R91 replaced with H, or K; D92 replaced with E; D93 replaced with E; I94 replaced with A, G, I, S, T, M, or V; L95 replaced with A, G, I, S, T, M, or V; Y96 replaced with F, or W; F97 replaced with W, or Y; S98 replaced with A, G, I, L, T, M, or V; I99 replaced with A, G, I, S, T, M, or V; R100 replaced with H, or K; N101 replaced with Q; G102 replaced with A, I, L, S, T, M, or V; T103 replaced with A, G, I, L, S, M, or V; H104 replaced with K, or R; V106 replaced with A, G, I, L, S, T, or M; F107 replaced with W, or Y; T108 replaced with A, G, I, L, S, M, or V; A109 replaced with G, I, L, S, T, M, or V; S110 replaced with A, G, I, L, T, M, or V; R111 replaced with H, or K; W112 replaced with F, or Y; M113 replaced with A, G, I, L, S, T, or V; V114 replaced with A, G, I, L, S, T, or M; Y115 replaced with F, or W; Y116 replaced with F, or W; L117 replaced with A, G, I, S, T, M, or V; K118 replaced with H, or R; S120 replaced with A, G, I, L, T, M, or V; S121 replaced with A, G, I, L, T, M, or V; K123 replaced with H, or R; H124 replaced with K, or R; V125 replaced with A, G, I, L, S, T, or M; R126 replaced with H, or K; F127 replaced with W, or Y; S128 replaced with A, G, I, L, T, M, or V; W129 replaced with F, or Y; H130 replaced with K, or R; Q131 replaced with N; D132 replaced with E; A133 replaced with G, I, L, S, T, M, or V; V134 replaced with A, G, I, L, S, T, or M; T135 replaced with A, G, I, L, S, M, or V; V136 replaced with A, G, I, L, S, T, or M; T137 replaced with A, G, I, L, S, M, or V; S139 replaced with A, G, I, L, T, M, or V; D140 replaced with E; L141 replaced with A, G, I, S, T, M, or V; S142 replaced with A, G, I, L, T, M, or V; Y143 replaced with F, or W; G144 replaced with A, I, L, S, T, M, or V; D145 replaced with E; L146 replaced with A,

G, I, S, T, M, or V; L147 replaced with A, G, I, S, T, M, or V; Y148 replaced with F, or W; E149 replaced with D; V150 replaced with A, G, I, L, S, T, or M; Q151 replaced with N; Y152 replaced with F, or W; R153 replaced with H, or K; S154 replaced with A, G, I, L, T, M, or V; F156 replaced with W, or Y; D157 replaced with E; T158 replaced with A, G, I, L, S, M, or V; E159 replaced with D; W160 replaced with F, or Y; Q161 replaced with N; S162 replaced with A, G, I, L, T, M, or V; K163 replaced with H, or R; Q164 replaced with N; E165 replaced with D; N166 replaced with Q; T167 replaced with A, G, I, L, S, M, or V; N169 replaced with Q; V170 replaced with A, G, I, L, S, T, or M; T171 replaced with A, G, I, L, S, M, or V; I172 replaced with A, G, L, S, T, M, or V; E173 replaced with D; G174 replaced with A, I, L, S, T, M, or V; L175 replaced with A, G, I, S, T, M, or V; D176 replaced with E; A177 replaced with G, I, L, S, T, M, or V; E178 replaced with D; K179 replaced with H, or R; Y181 replaced with F, or W; S182 replaced with A, G, I, L, T, M, or V; F183 replaced with W, or Y; W184 replaced with F, or Y; V185 replaced with A, G, I, L, S, T, or M; R186 replaced with H, or K; V187 replaced with A, G, I, L, S, T, or M; K188 replaced with H, or R; A189 replaced with G, I, L, S, T, M, or V; M190 replaced with A, G, I, L, S, T, or V; E191 replaced with D; D192 replaced with E; V193 replaced with A, G, I, L, S, T, or M; Y194 replaced with F, or W; G195 replaced with A, I, L, S, T, M, or V; D197 replaced with E; T198 replaced with A, G, I, L, S, M, or V; Y199 replaced with F, or W; S201 replaced with A, G, I, L, T, M, or V; D202 replaced with E; W203 replaced with F, or Y; S204 replaced with A, G, I, L, T, M, or V; E205 replaced with D; V206 replaced with A, G, I, L, S, T, or M; T207 replaced with A, G, I, L, S, M, or V; W209 replaced with F, or Y; Q210 replaced with N; R211 replaced with H, or K; G212 replaced with A, I, L, S, T, M, or V; E213 replaced with D; I214 replaced with A, G, L, S, T, M, or V; R215 replaced with H, or K; D216 replaced with E; A217 replaced with G, I, L, S, T, M, or V; A219 replaced with G, I, L, S, T, M, or V; E220 replaced with D; T221 replaced with A, G, I, L, S, M, or V; T223 replaced with A, G, I, L, S, M, or V; K226 replaced with H, or R; K228 replaced with H, or R; L229 replaced with A, G, I, S, T, M, or V; S230 replaced with A, G, I, L, T, M, or V; K231 replaced with H, or R; F232 replaced with W, or Y; I233 replaced with A, G, L, S, T, M, or V; L234 replaced with A, G, I, S, T, M, or V; I235 replaced with A, G, L, S, T, M, or V; S236 replaced with A, G, I, L, T, M, or V; S237 replaced with A, G, I, L, T, M, or V; L238 replaced with A, G, I, S, T, M, or V; A239 replaced with G, I, L, S, T, M, or V; I240 replaced with A, G, L, S, T, M, or V; L241 replaced with A, G, I, S, T, M, or V; L242 replaced with A, G, I, S, T, M, or V; M243 replaced with A, G, I, L, S, T, or V; V244

replaced with A, G, I, L, S, T, or M; S245 replaced with A, G, I, L, T, M, or V; L246 replaced with A, G, I, S, T, M, or V; L247 replaced with A, G, I, S, T, M, or V; L248 replaced with A, G, I, S, T, M, or V; L249 replaced with A, G, I, S, T, M, or V; S250 replaced with A, G, I, L, T, M, or V; L251 replaced with A, G, I, S, T, M, or V; W252 replaced with F, or Y; K253 replaced with H, or R; L254 replaced with A, G, I, S, T, M, or V; W255 replaced with F, or Y; R256 replaced with H, or K; V257 replaced with A, G, I, L, S, T, or M; K258 replaced with H, or R; K259 replaced with H, or R; F260 replaced with W, or Y; L261 replaced with A, G, I, S, T, M, or V; I262 replaced with A, G, L, S, T, M, or V; S264 replaced with A, G, I, L, T, M, or V; V265 replaced with A, G, I, L, S, T, or M; D267 replaced with E; K269 replaced with H, or R; S270 replaced with A, G, I, L, T, M, or V; I271 replaced with A, G, L, S, T, M, or V; F272 replaced with W, or Y; G274 replaced with A, I, L, S, T, M, or V; L275 replaced with A, G, I, S, T, M, or V; F276 replaced with W, or Y; E277 replaced with D; I278 replaced with A, G, L, S, T, M, or V; H279 replaced with K, or R; Q280 replaced with N; G281 replaced with A, I, L, S, T, M, or V; N282 replaced with Q; F283 replaced with W, or Y; Q284 replaced with N; E285 replaced with D; W286 replaced with F, or Y; I287 replaced with A, G, L, S, T, M, or V; T288 replaced with A, G, I, L, S, M, or V; D289 replaced with E; T290 replaced with A, G, I, L, S, M, or V; Q291 replaced with N; N292 replaced with Q; V293 replaced with A, G, I, L, S, T, or M; A294 replaced with G, I, L, S, T, M, or V; H295 replaced with K, or R; L296 replaced with A, G, I, S, T, M, or V; H297 replaced with K, or R; K298 replaced with H, or R; M299 replaced with A, G, I, L, S, T, or V; A300 replaced with G, I, L, S, T, M, or V; G301 replaced with A, I, L, S, T, M, or V; A302 replaced with G, I, L, S, T, M, or V; E303 replaced with D; Q304 replaced with N; E305 replaced with D; S306 replaced with A, G, I, L, T, M, or V; G307 replaced with A, I, L, S, T, M, or V; E309 replaced with D; E310 replaced with D; L312 replaced with A, G, I, S, T, M, or V; V313 replaced with A, G, I, L, S, T, or M; V314 replaced with A, G, I, L, S, T, or M; Q315 replaced with N; L316 replaced with A, G, I, S, T, M, or V; A317 replaced with G, I, L, S, T, M, or V; K318 replaced with H, or R; T319 replaced with A, G, I, L, S, M, or V; E320 replaced with D; A321 replaced with G, I, L, S, T, M, or V; E322 replaced with D; S323 replaced with A, G, I, L, T, M, or V; R325 replaced with H, or K; M326 replaced with A, G, I, L, S, T, or V; L327 replaced with A, G, I, S, T, M, or V; D328 replaced with E; Q330 replaced with N; T331 replaced with A, G, I, L, S, M, or V; E332 replaced with D; E333 replaced with D; K334 replaced with H, or R; E335 replaced with D; A336 replaced with G, I, L, S, T, M, or V; S337 replaced with A, G, I, L, T, M, or V; G338 replaced with A, I, L, S,

T, M, or V; G339 replaced with A, I, L, S, T, M, or V; S340 replaced with A, G, I, L, T, M, or V; L341 replaced with A, G, I, S, T, M, or V; Q342 replaced with N; L343 replaced with A, G, I, S, T, M, or V; H345 replaced with K, or R; Q346 replaced with N; L348 replaced with A, G, I, S, T, M, or V; Q349 replaced with N; G350 replaced with A, I, L, S, T, M, or V; G351 replaced with A, I, L, S, T, M, or V; D352 replaced with E; V353 replaced with A, G, I, L, S, T, or M; V354 replaced with A, G, I, L, S, T, or M; T355 replaced with A, G, I, L, S, M, or V; I356 replaced with A, G, L, S, T, M, or V; G357 replaced with A, I, L, S, T, M, or V; G358 replaced with A, I, L, S, T, M, or V; F359 replaced with W, or Y; T360 replaced with A, G, I, L, S, M, or V; F361 replaced with W, or Y; V362 replaced with A, G, I, L, S, T, or M; M363 replaced with A, G, I, L, S, T, or V; N364 replaced with Q; D365 replaced with E; R366 replaced with H, or K; S367 replaced with A, G, I, L, T, M, or V; Y368 replaced with F, or W; V369 replaced with A, G, I, L, S, T, or M; A370 replaced with G, I, L, S, T, M, or V; L371 replaced with A, G, I, S, T, M, or V.

[0095] The resulting constructs can be routinely screened for activities or functions described throughout the specification and known in the art. Preferably, the resulting constructs have an increased CRCGCL activity or function, while the remaining CRCGCL activities or functions are maintained. More preferably, the resulting constructs have more than one increased CRCGCL activity or function, while the remaining CRCGCL activities or functions are maintained.

[0096] Besides conservative amino acid substitution, variants of CRCGCL include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

[0097] For example, CRCGCL polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's

immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

[0098] For example, preferred non-conservative substitutions of CRCGCL include: M1 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G2 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R3 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L4 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V5 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L6 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L7 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W8 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G9 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A10 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A11 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V12 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F13 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L14 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L15 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G16 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G17 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W18 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; M19 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A20 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L21 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G22 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q23 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G24 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G25 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A26 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A27 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E28 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G29 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V30 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q31 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I32 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q33 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I34 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I35 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y36 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F37 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N38 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L39 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E40 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T41 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V42 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q43 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V44

replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T45 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W46 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N47 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A48 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S49 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K50 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y51 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S52 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R53 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T54 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N55 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L56 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T57 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F58 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; H59 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y60 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R61 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F62 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N63 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G64 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D65 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E66 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A67 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y68 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D69 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q70 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; C71 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; T72 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N73 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y74 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L75 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L76 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q77 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E78 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G79 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H80 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T81 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S82 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G83 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C84 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L85 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L86 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D87 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A88 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E89 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q90

replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R91 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D92 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D93 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I94 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L95 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y96 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F97 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S98 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I99 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R100 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N101 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G102 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T103 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H104 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P105 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; V106 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F107 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T108 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A109 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S110 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R111 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; W112 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; M113 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V114 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y115 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Y116 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L117 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K118 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P119 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S120 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S121 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P122 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K123 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H124 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V125 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R126 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F127 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S128 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W129 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; H130 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q131 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D132 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A133 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V134 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T135 replaced with D, E, H, K, R,



N, Q, F, W, Y, P, or C; V136 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T137 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C138 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; S139 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D140 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L141 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S142 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y143 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G144 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D145 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L146 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L147 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y148 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; E149 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V150 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q151 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y152 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R153 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S154 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P155 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; F156 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D157 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T158 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E159 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; W160 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q161 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S162 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K163 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q164 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E165 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N166 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T167 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C168 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; N169 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V170 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T171 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I172 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E173 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G174 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L175 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D176 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A177 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E178 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K179 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C180 replaced with D, E, H, K, R,

A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Y181 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S182 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F183 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; W184 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V185 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R186 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V187 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K188 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A189 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M190 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E191 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D192 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V193 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y194 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G195 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P196 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; D197 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T198 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y199 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; P200 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S201 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D202 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; W203 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S204 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E205 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V206 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T207 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C208 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; W209 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q210 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R211 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G212 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E213 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I214 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R215 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D216 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A217 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C218 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; A219 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E220 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T221 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P222 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T223 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P224 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q,

F, W, Y, or C; P225 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K226 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P227 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K228 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L229 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S230 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K231 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F232 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I233 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L234 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I235 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S236 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S237 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L238 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A239 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I240 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L241 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L242 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M243 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V244 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S245 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L246 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L247 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L248 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L249 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S250 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L251 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W252 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K253 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L254 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W255 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R256 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V257 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K258 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K259 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F260 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L261 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I262 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P263 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S264 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V265 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P266 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; D267 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P268 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K269 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S270 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I271 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F272

replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; P273 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G274 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L275 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F276 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; E277 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I278 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H279 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q280 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G281 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N282 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; F283 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q284 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E285 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; W286 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I287 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T288 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D289 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T290 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q291 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N292 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V293 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A294 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H295 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L296 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H297 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K298 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M299 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A300 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G301 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A302 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E303 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q304 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E305 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S306 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G307 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P308 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E309 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E310 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P311 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L312 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V313 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V314 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q315 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L316 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C;

A317 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K318 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T319 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E320 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A321 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E322 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S323 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P324 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R325 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M326 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L327 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D328 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P329 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Q330 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T331 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E332 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E333 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K334 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E335 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A336 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S337 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G338 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G339 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S340 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L341 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q342 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L343 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P344 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; H345 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q346 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P347 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L348 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q349 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G350 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G351 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D352 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V353 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V354 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T355 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I356 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G357 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G358 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F359 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T360 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F361 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V362 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M363 replaced

with D, E, H, K, R, N, Q, F, W, Y, P, or C; N364 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D365 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R366 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S367 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y368 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V369 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A370 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L371 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C.

[0099] The resulting constructs can be routinely screened for activities or functions described throughout the specification and known in the art. Preferably, the resulting constructs have loss of a CRCGCL activity or function, while the remaining CRCGCL activities or functions are maintained. More preferably, the resulting constructs have more than one loss of CRCGCL activity or function, while the remaining CRCGCL activities or functions are maintained.

[0100] Additionally, more than one amino acid (e.g., 2, 3, 4, 5, 6, 7, 8, 9 and 10) can be replaced with the substituted amino acids as described above (either conservative or nonconservative). The substituted amino acids can occur in the full length, mature, or proprotein form of CRCGCL protein, as well as the N- and C- terminal deletion mutants, having the general formula  $m-n$ ,  $m-n^1$ ,  $m^1-n$  and/or  $m^1-n^1$ , listed below.

[0101] A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of a CRCGCL polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of a CRCGCL polypeptide, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of Figures 1A-1B or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

#### **Polynucleotide and Polypeptide Fragments**

[0102] The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having, for example, the nucleotide sequence of the deposited cDNA (clone HTAEK53), a nucleotide sequence encoding the polypeptide sequence encoded by the deposited cDNA, a nucleotide sequence encoding the polypeptide sequence depicted in Figures 1A-1B (SEQ ID NO:2), the nucleotide sequence shown in Figures 1A-1B (SEQ ID NO:1), or the complementary strand thereto, is intended fragments at least 15 nt, and more preferably at least about 20 nt, still more preferably at least 30 nt, and even more preferably, at least about 40, 50, 100, 150, 200, 250, 300, 325, 350, 375, 400, 450, 500, 550, or 600 nt in length. These fragments have numerous uses that include, but are not limited to, diagnostic probes and primers as discussed herein. Of course, larger fragments, such as those of 501-1500 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequences of the deposited cDNA (clone HTAEK53) or as shown in Figures 1A-1B (SEQ ID NO:1). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from, for example, the nucleotide sequence of the deposited cDNA, or the nucleotide sequence as shown in Figures 1A-1B (SEQ ID NO:1).

[0103] Moreover, representative examples of CRCGCL polynucleotide fragments include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, and/or 2001 to the end of SEQ ID NO:1 or the complementary strand thereto, or the cDNA contained in the deposited clone. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

[0104] Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a CRCGCL functional activity. By a polypeptide demonstrating a CRCGCL "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) CRCGCL protein. Such functional activities include, but are not limited to, biological activity [ability to promote B cell lymphopoiesis; ability to costimulate thymocytes or mature T cells, ability

to promote the proliferation, differentiation, and/or chemotaxis of T cells; ability to promote the release of T-cell attracting chemokines from monocytes; ability to enhance the T cell stimulatory capacity of CD11c+ subset of dendritic cells; and/or ability to activate STAT5B], antigenicity [ability to bind (or compete with a CRCGCL polypeptide for binding) to an anti-CRCGCL antibody], immunogenicity (ability to generate antibody which binds to a CRCGCL polypeptide), ability to form multimers with CRCGCL polypeptides of the invention, ability to form multimers with other type I cytokine receptor chains (e.g., IL-7 alpha chain) and/or ability to bind to a ligand for a CRCGCL polypeptide (e.g., TSLP).

[0105] The functional activity of CRCGCL polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

[0106] For example, in one embodiment where one is assaying for the ability to bind or compete with full-length CRCGCL polypeptide for binding to anti-CRCGCL antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

[0107] In another embodiment, where a CRCGCL ligand is identified (e.g., TSLP), or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated (e.g., complex with another type I cytokine receptor chain, for example, IL-7R alpha chain), binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, Microbiol. Rev. 59:94-123. In another embodiment, physiological correlates of CRCGCL binding to its substrates (signal transduction) can be assayed.



[0108] For example, in one embodiment, where one is assaying for physiological correlates of signal transduction resulting from CRCGCL binding to its ligand (e.g., TSLP), various assays known in the art can be used, including but not limited to, a 293T reconstitution assay (see, e.g., Example 54). In a preferred embodiment, 293T cells are transfected with cDNA encoding CRCGCL, IL-7R alpha chain, Jax2 and STAT5 and stimulated with human TSLP.

[0109] In a further embodiment, antagonists of CRCGCL (including, but not limited to, antibodies directed against CRCGCL and soluble forms of CRCGCL) may be assayed in various assays known in the art can be used, including but not limited to, the 293T reconstitution assay described above (see, e.g., Example 54).

[0110] In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of CRCGCL polypeptides and fragments, variants derivatives and analogs thereof to elicit CRCGCL related biological activity (either *in vitro* or *in vivo*). Other methods will be known to the skilled artisan and are within the scope of the invention.

[0111] The present invention is further directed to fragments of the CRCGCL polypeptide described herein. By a fragment of an isolated the CRCGCL polypeptide, for example, encoded by the deposited cDNA (clone HTAEK53), the polypeptide sequence encoded by the deposited cDNA, the polypeptide sequence depicted in Figures 1A-1B (SEQ ID NO:2), is intended to encompass polypeptide fragments contained in SEQ ID NO:2 or encoded by the cDNA contained in the deposited clone. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, or 281 to the end of the coding region. Moreover, polypeptide fragments can be at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

[0112] Even if deletion of one or more amino acids from the N-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities (e.g., biological activities [ability to promote B cell lymphopoiesis;

ability to costimulate thymocytes or mature T cells, ability to promote the proliferation, differentiation, and/or chemotaxis of T cells; ability to promote the release of T-cell attracting chemokines from monocytes; ability to enhance the T cell stimulatory capacity of CD11c+ subset of dendritic cells; and/or ability to activate STAT5B]; antigenicity [ability to bind (or compete with a CRCGCL polypeptide for binding) to an anti-CRCGCL antibody], immunogenicity (ability to generate antibody which binds to a CRCGCL polypeptide), ability to form multimers with CRCGCL polypeptides of the invention, ability to form multimers with other type I cytokine receptor chains (e.g., IL-7R alpha chain) and/or ability to bind to a ligand for a CRCGCL polypeptide (e.g., TSLP)] may still be retained. For example, the ability of shortened CRCGCL muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that an CRCGCL mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six CRCGCL amino acid residues may often evoke an immune response.

[0113] Accordingly, polypeptide fragments include the secreted CRCGCL protein as well as the mature form. Further preferred polypeptide fragments include the secreted CRCGCL protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted CRCGCL polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted CRCGCL protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotide fragments encoding these CRCGCL polypeptide fragments are also preferred.

[0114] Particularly, N-terminal deletions of the CRCGCL polypeptide can be described by the general formula m-371, where m is an integer from 2 to 370, where m corresponds to the position of the amino acid residue identified in SEQ ID NO:2. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of N-terminal deletions of the

CRCGCL polypeptide of the invention shown as SEQ ID NO:2, including polypeptides comprising the amino acid sequence of residues: G-2 to L-371; R-3 to L-371; L-4 to L-371; V-5 to L-371; L-6 to L-371; L-7 to L-371; W-8 to L-371; G-9 to L-371; A-10 to L-371; A-11 to L-371; V-12 to L-371; F-13 to L-371; L-14 to L-371; L-15 to L-371; G-16 to L-371; G-17 to L-371; W-18 to L-371; M-19 to L-371; A-20 to L-371; L-21 to L-371; G-22 to L-371; Q-23 to L-371; G-24 to L-371; G-25 to L-371; A-26 to L-371; A-27 to L-371; E-28 to L-371; G-29 to L-371; V-30 to L-371; Q-31 to L-371; I-32 to L-371; Q-33 to L-371; I-34 to L-371; I-35 to L-371; Y-36 to L-371; F-37 to L-371; N-38 to L-371; L-39 to L-371; E-40 to L-371; T-41 to L-371; V-42 to L-371; Q-43 to L-371; V-44 to L-371; T-45 to L-371; W-46 to L-371; N-47 to L-371; A-48 to L-371; S-49 to L-371; K-50 to L-371; Y-51 to L-371; S-52 to L-371; R-53 to L-371; T-54 to L-371; N-55 to L-371; L-56 to L-371; T-57 to L-371; F-58 to L-371; H-59 to L-371; Y-60 to L-371; R-61 to L-371; F-62 to L-371; N-63 to L-371; G-64 to L-371; D-65 to L-371; E-66 to L-371; A-67 to L-371; Y-68 to L-371; D-69 to L-371; Q-70 to L-371; C-71 to L-371; T-72 to L-371; N-73 to L-371; Y-74 to L-371; L-75 to L-371; L-76 to L-371; Q-77 to L-371; E-78 to L-371; G-79 to L-371; H-80 to L-371; T-81 to L-371; S-82 to L-371; G-83 to L-371; C-84 to L-371; L-85 to L-371; L-86 to L-371; D-87 to L-371; A-88 to L-371; E-89 to L-371; Q-90 to L-371; R-91 to L-371; D-92 to L-371; D-93 to L-371; I-94 to L-371; L-95 to L-371; Y-96 to L-371; F-97 to L-371; S-98 to L-371; I-99 to L-371; R-100 to L-371; N-101 to L-371; G-102 to L-371; T-103 to L-371; H-104 to L-371; P-105 to L-371; V-106 to L-371; F-107 to L-371; T-108 to L-371; A-109 to L-371; S-110 to L-371; R-111 to L-371; W-112 to L-371; M-113 to L-371; V-114 to L-371; Y-115 to L-371; Y-116 to L-371; L-117 to L-371; K-118 to L-371; P-119 to L-371; S-120 to L-371; S-121 to L-371; P-122 to L-371; K-123 to L-371; H-124 to L-371; V-125 to L-371; R-126 to L-371; F-127 to L-371; S-128 to L-371; W-129 to L-371; H-130 to L-371; Q-131 to L-371; D-132 to L-371; A-133 to L-371; V-134 to L-371; T-135 to L-371; V-136 to L-371; T-137 to L-371; C-138 to L-371; S-139 to L-371; D-140 to L-371; L-141 to L-371; S-142 to L-371; Y-143 to L-371; G-144 to L-371; D-145 to L-371; L-146 to L-371; L-147 to L-371; Y-148 to L-371; E-149 to L-371; V-150 to L-371; Q-151 to L-371; Y-152 to L-371; R-153 to L-371; S-154 to L-371; P-155 to L-371; F-156 to L-371; D-157 to L-371; T-158 to L-371; E-159 to L-371; W-160 to L-371; Q-161 to L-371; S-162 to L-371; K-163 to L-371; Q-164 to L-371; E-165 to L-371; N-166 to L-371; T-167 to L-371; C-168 to L-371; N-169 to L-371; V-170 to L-371; T-171 to L-371; I-172 to L-371; E-173 to L-371; G-174 to L-371; L-175 to L-371; D-176 to L-371; A-177 to L-371; E-178 to L-371; K-179 to L-371; C-180 to L-371; Y-181 to L-371; S-182 to L-371; F-183 to

L-371; W-184 to L-371; V-185 to L-371; R-186 to L-371; V-187 to L-371; K-188 to L-371; A-189 to L-371; M-190 to L-371; E-191 to L-371; D-192 to L-371; V-193 to L-371; Y-194 to L-371; G-195 to L-371; P-196 to L-371; D-197 to L-371; T-198 to L-371; Y-199 to L-371; P-200 to L-371; S-201 to L-371; D-202 to L-371; W-203 to L-371; S-204 to L-371; E-205 to L-371; V-206 to L-371; T-207 to L-371; C-208 to L-371; W-209 to L-371; Q-210 to L-371; R-211 to L-371; G-212 to L-371; E-213 to L-371; I-214 to L-371; R-215 to L-371; D-216 to L-371; A-217 to L-371; C-218 to L-371; A-219 to L-371; E-220 to L-371; T-221 to L-371; P-222 to L-371; T-223 to L-371; P-224 to L-371; P-225 to L-371; K-226 to L-371; P-227 to L-371; K-228 to L-371; L-229 to L-371; S-230 to L-371; K-231 to L-371; F-232 to L-371; I-233 to L-371; L-234 to L-371; I-235 to L-371; S-236 to L-371; S-237 to L-371; L-238 to L-371; A-239 to L-371; I-240 to L-371; L-241 to L-371; L-242 to L-371; M-243 to L-371; V-244 to L-371; S-245 to L-371; L-246 to L-371; L-247 to L-371; L-248 to L-371; L-249 to L-371; S-250 to L-371; L-251 to L-371; W-252 to L-371; K-253 to L-371; L-254 to L-371; W-255 to L-371; R-256 to L-371; V-257 to L-371; K-258 to L-371; K-259 to L-371; F-260 to L-371; L-261 to L-371; I-262 to L-371; P-263 to L-371; S-264 to L-371; V-265 to L-371; P-266 to L-371; D-267 to L-371; P-268 to L-371; K-269 to L-371; S-270 to L-371; I-271 to L-371; F-272 to L-371; P-273 to L-371; G-274 to L-371; L-275 to L-371; F-276 to L-371; E-277 to L-371; I-278 to L-371; H-279 to L-371; Q-280 to L-371; G-281 to L-371; N-282 to L-371; F-283 to L-371; Q-284 to L-371; E-285 to L-371; W-286 to L-371; I-287 to L-371; T-288 to L-371; D-289 to L-371; T-290 to L-371; Q-291 to L-371; N-292 to L-371; V-293 to L-371; A-294 to L-371; H-295 to L-371; L-296 to L-371; H-297 to L-371; K-298 to L-371; M-299 to L-371; A-300 to L-371; G-301 to L-371; A-302 to L-371; E-303 to L-371; Q-304 to L-371; E-305 to L-371; S-306 to L-371; G-307 to L-371; P-308 to L-371; E-309 to L-371; E-310 to L-371; P-311 to L-371; L-312 to L-371; V-313 to L-371; V-314 to L-371; Q-315 to L-371; L-316 to L-371; A-317 to L-371; K-318 to L-371; T-319 to L-371; E-320 to L-371; A-321 to L-371; E-322 to L-371; S-323 to L-371; P-324 to L-371; R-325 to L-371; M-326 to L-371; L-327 to L-371; D-328 to L-371; P-329 to L-371; Q-330 to L-371; T-331 to L-371; E-332 to L-371; E-333 to L-371; K-334 to L-371; E-335 to L-371; A-336 to L-371; S-337 to L-371; G-338 to L-371; G-339 to L-371; S-340 to L-371; L-341 to L-371; Q-342 to L-371; L-343 to L-371; P-344 to L-371; H-345 to L-371; Q-346 to L-371; P-347 to L-371; L-348 to L-371; Q-349 to L-371; G-350 to L-371; G-351 to L-371; D-352 to L-371; V-353 to L-371; V-354 to L-371; T-355 to L-371; I-356 to L-371; G-357 to L-371; G-358 to L-371; F-359 to L-371; T-360 to L-371; F-361 to L-371; V-362 to L-371; M-363 to L-371; N-364 to L-371; D-

365 to L- 371; R-366 to L-371; of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0115] Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind CRCGCL ligand) may still be retained. For example the ability of the shortened CRCGCL mutein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that an CRCGCL mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six CRCGCL amino acid residues may often evoke an immune response.

[0116] Accordingly, the present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the CRCGCL polypeptide shown in Figures 1A-1B (SEQ ID NO:2), as described by the general formula 1-n, where n is an integer from 2 to 371, where n corresponds to the position of amino acid residue identified in SEQ ID NO:2. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of C-terminal deletions of the CRCGCL polypeptide of the invention shown as SEQ ID NO:2 include polypeptides comprising the amino acid sequence of residues: M-1 to A-370; M-1 to V-369; M-1 to Y-368; M-1 to S-367; M-1 to R-366; M-1 to D-365; M-1 to N-364; M-1 to M-363; M-1 to V-362; M-1 to F-361; M-1 to T-360; M-1 to F-359; M-1 to G-358; M-1 to G-357; M-1 to I-356; M-1 to T-355; M-1 to V-354; M-1 to V-353; M-1 to D-352; M-1 to G-351; M-1 to G-350; M-1 to Q-349; M-1 to L-348; M-1 to P-347; M-1 to Q-346; M-1 to H-345; M-1 to P-344; M-1 to L-343; M-1 to Q-342; M-1 to L-341; M-1 to S-340; M-1 to G-339; M-1 to G-338; M-1 to S-337; M-1 to A-336; M-1 to E-335; M-1 to K-334; M-1 to E-333; M-1 to E-332; M-1 to T-331; M-1 to Q-330; M-1 to P-329; M-1 to D-328; M-1 to L-327; M-1 to M-326; M-1 to R-325; M-1 to P-324; M-1 to S-323; M-1 to E-322; M-1 to A-321; M-1 to E-320; M-1 to T-319; M-1 to K-318; M-1 to A-317; M-1 to L-316; M-1 to Q-315; M-1 to V-314; M-1 to V-313; M-1 to L-312; M-1 to P-

311; M-1 to E-310; M-1 to E-309; M-1 to P-308; M-1 to G-307; M-1 to S-306; M-1 to E-305; M-1 to Q-304; M-1 to E-303; M-1 to A-302; M-1 to G-301; M-1 to A-300; M-1 to M-299; M-1 to K-298; M-1 to H-297; M-1 to L-296; M-1 to H-295; M-1 to A-294; M-1 to V-293; M-1 to N-292; M-1 to Q-291; M-1 to T-290; M-1 to D-289; M-1 to T-288; M-1 to I-287; M-1 to W-286; M-1 to E-285; M-1 to Q-284; M-1 to F-283; M-1 to N-282; M-1 to G-281; M-1 to Q-280; M-1 to H-279; M-1 to I-278; M-1 to E-277; M-1 to F-276; M-1 to L-275; M-1 to G-274; M-1 to P-273; M-1 to F-272; M-1 to I-271; M-1 to S-270; M-1 to K-269; M-1 to P-268; M-1 to D-267; M-1 to P-266; M-1 to V-265; M-1 to S-264; M-1 to P-263; M-1 to I-262; M-1 to L-261; M-1 to F-260; M-1 to K-259; M-1 to K-258; M-1 to V-257; M-1 to R-256; M-1 to W-255; M-1 to L-254; M-1 to K-253; M-1 to W-252; M-1 to L-251; M-1 to S-250; M-1 to L-249; M-1 to L-248; M-1 to L-247; M-1 to L-246; M-1 to S-245; M-1 to V-244; M-1 to M-243; M-1 to L-242; M-1 to L-241; M-1 to I-240; M-1 to A-239; M-1 to L-238; M-1 to S-237; M-1 to S-236; M-1 to I-235; M-1 to L-234; M-1 to I-233; M-1 to F-232; M-1 to K-231; M-1 to S-230; M-1 to L-229; M-1 to K-228; M-1 to P-227; M-1 to K-226; M-1 to P-225; M-1 to P-224; M-1 to T-223; M-1 to P-222; M-1 to T-221; M-1 to E-220; M-1 to A-219; M-1 to C-218; M-1 to A-217; M-1 to D-216; M-1 to R-215; M-1 to I-214; M-1 to E-213; M-1 to G-212; M-1 to R-211; M-1 to Q-210; M-1 to W-209; M-1 to C-208; M-1 to T-207; M-1 to V-206; M-1 to E-205; M-1 to S-204; M-1 to W-203; M-1 to D-202; M-1 to S-201; M-1 to P-200; M-1 to Y-199; M-1 to T-198; M-1 to D-197; M-1 to P-196; M-1 to G-195; M-1 to Y-194; M-1 to V-193; M-1 to D-192; M-1 to E-191; M-1 to M-190; M-1 to A-189; M-1 to K-188; M-1 to V-187; M-1 to R-186; M-1 to V-185; M-1 to W-184; M-1 to F-183; M-1 to S-182; M-1 to Y-181; M-1 to C-180; M-1 to K-179; M-1 to E-178; M-1 to A-177; M-1 to D-176; M-1 to L-175; M-1 to G-174; M-1 to E-173; M-1 to I-172; M-1 to T-171; M-1 to V-170; M-1 to N-169; M-1 to C-168; M-1 to T-167; M-1 to N-166; M-1 to E-165; M-1 to Q-164; M-1 to K-163; M-1 to S-162; M-1 to Q-161; M-1 to W-160; M-1 to E-159; M-1 to T-158; M-1 to D-157; M-1 to F-156; M-1 to P-155; M-1 to S-154; M-1 to R-153; M-1 to Y-152; M-1 to Q-151; M-1 to V-150; M-1 to E-149; M-1 to Y-148; M-1 to L-147; M-1 to L-146; M-1 to D-145; M-1 to G-144; M-1 to Y-143; M-1 to S-142; M-1 to L-141; M-1 to D-140; M-1 to S-139; M-1 to C-138; M-1 to T-137; M-1 to V-136; M-1 to T-135; M-1 to V-134; M-1 to A-133; M-1 to D-132; M-1 to Q-131; M-1 to H-130; M-1 to W-129; M-1 to S-128; M-1 to F-127; M-1 to R-126; M-1 to V-125; M-1 to H-124; M-1 to K-123; M-1 to P-122; M-1 to S-121; M-1 to S-120; M-1 to P-119; M-1 to K-118; M-1 to L-117; M-1 to Y-116; M-1 to Y-115; M-1 to V-114; M-1 to M-113; M-1 to W-

112; M-1 to R-111; M-1 to S-110; M-1 to A-109; M-1 to T-108; M-1 to F-107; M-1 to V-106; M-1 to P-105; M-1 to H-104; M-1 to T-103; M-1 to G-102; M-1 to N-101; M-1 to R-100; M-1 to I-99; M-1 to S-98; M-1 to F-97; M-1 to Y-96; M-1 to L-95; M-1 to I-94; M-1 to D-93; M-1 to D-92; M-1 to R-91; M-1 to Q-90; M-1 to E-89; M-1 to A-88; M-1 to D-87; M-1 to L-86; M-1 to L-85; M-1 to C-84; M-1 to G-83; M-1 to S-82; M-1 to T-81; M-1 to H-80; M-1 to G-79; M-1 to E-78; M-1 to Q-77; M-1 to L-76; M-1 to L-75; M-1 to Y-74; M-1 to N-73; M-1 to T-72; M-1 to C-71; M-1 to Q-70; M-1 to D-69; M-1 to Y-68; M-1 to A-67; M-1 to E-66; M-1 to D-65; M-1 to G-64; M-1 to N-63; M-1 to F-62; M-1 to R-61; M-1 to Y-60; M-1 to H-59; M-1 to F-58; M-1 to T-57; M-1 to L-56; M-1 to N-55; M-1 to T-54; M-1 to R-53; M-1 to S-52; M-1 to Y-51; M-1 to K-50; M-1 to S-49; M-1 to A-48; M-1 to N-47; M-1 to W-46; M-1 to T-45; M-1 to V-44; M-1 to Q-43; M-1 to V-42; M-1 to T-41; M-1 to E-40; M-1 to L-39; M-1 to N-38; M-1 to F-37; M-1 to Y-36; M-1 to I-35; M-1 to I-34; M-1 to Q-33; M-1 to I-32; M-1 to Q-31; M-1 to V-30; M-1 to G-29; M-1 to E-28; M-1 to A-27; M-1 to A-26; M-1 to G-25; M-1 to G-24; M-1 to Q-23; M-1 to G-22; M-1 to L-21; M-1 to A-20; M-1 to M-19; M-1 to W-18; M-1 to G-17; M-1 to G-16; M-1 to L-15; M-1 to L-14; M-1 to F-13; M-1 to V-12; M-1 to A-11; M-1 to A-10; M-1 to G-9; M-1 to W-8; M-1 to L-7; of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0117] In addition, any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted CRCGCL polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO:2, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0118] Accordingly, N-terminal deletion mutants comprising the soluble domain of SEQ ID NO:2 are also contemplated and can be described by the general formula  $m^1$ -231, where  $m^1$  is an integer from 2 to 226, where  $m^1$  corresponds to the position of the amino acid residue identified in SEQ ID NO:2. In particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of N-terminal deletions of the CRCGCL polypeptide of the invention shown as SEQ ID NO:2, including polypeptides comprising the amino acid sequence of residues: G-2 to K-231; R-3 to K-231; L-4 to K-231; V-5 to K-231; L-6 to K-231; L-7 to K-231; W-8 to K-231; G-9 to K-231; A-10 to K-231; A-11 to K-231; V-12 to K-

231; F-13 to K-231; L-14 to K-231; L-15 to K-231; G-16 to K-231; G-17 to K-231; W-18 to K-231; M-19 to K-231; A-20 to K-231; L-21 to K-231; G-22 to K-231; Q-23 to K-231; G-24 to K-231; G-25 to K-231; A-26 to K-231; A-27 to K-231; E-28 to K-231; G-29 to K-231; V-30 to K-231; Q-31 to K-231; I-32 to K-231; Q-33 to K-231; I-34 to K-231; I-35 to K-231; Y-36 to K-231; F-37 to K-231; N-38 to K-231; L-39 to K-231; E-40 to K-231; T-41 to K-231; V-42 to K-231; Q-43 to K-231; V-44 to K-231; T-45 to K-231; W-46 to K-231; N-47 to K-231; A-48 to K-231; S-49 to K-231; K-50 to K-231; Y-51 to K-231; S-52 to K-231; R-53 to K-231; T-54 to K-231; N-55 to K-231; L-56 to K-231; T-57 to K-231; F-58 to K-231; H-59 to K-231; Y-60 to K-231; R-61 to K-231; F-62 to K-231; N-63 to K-231; G-64 to K-231; D-65 to K-231; E-66 to K-231; A-67 to K-231; Y-68 to K-231; D-69 to K-231; Q-70 to K-231; C-71 to K-231; T-72 to K-231; N-73 to K-231; Y-74 to K-231; L-75 to K-231; L-76 to K-231; Q-77 to K-231; E-78 to K-231; G-79 to K-231; H-80 to K-231; T-81 to K-231; S-82 to K-231; G-83 to K-231; C-84 to K-231; L-85 to K-231; L-86 to K-231; D-87 to K-231; A-88 to K-231; E-89 to K-231; Q-90 to K-231; R-91 to K-231; D-92 to K-231; D-93 to K-231; I-94 to K-231; L-95 to K-231; Y-96 to K-231; F-97 to K-231; S-98 to K-231; I-99 to K-231; R-100 to K-231; N-101 to K-231; G-102 to K-231; T-103 to K-231; H-104 to K-231; P-105 to K-231; V-106 to K-231; F-107 to K-231; T-108 to K-231; A-109 to K-231; S-110 to K-231; R-111 to K-231; W-112 to K-231; M-113 to K-231; V-114 to K-231; Y-115 to K-231; Y-116 to K-231; L-117 to K-231; K-118 to K-231; P-119 to K-231; S-120 to K-231; S-121 to K-231; P-122 to K-231; K-123 to K-231; H-124 to K-231; V-125 to K-231; R-126 to K-231; F-127 to K-231; S-128 to K-231; W-129 to K-231; H-130 to K-231; Q-131 to K-231; D-132 to K-231; A-133 to K-231; V-134 to K-231; T-135 to K-231; V-136 to K-231; T-137 to K-231; C-138 to K-231; S-139 to K-231; D-140 to K-231; L-141 to K-231; S-142 to K-231; Y-143 to K-231; G-144 to K-231; D-145 to K-231; L-146 to K-231; L-147 to K-231; Y-148 to K-231; E-149 to K-231; V-150 to K-231; Q-151 to K-231; Y-152 to K-231; R-153 to K-231; S-154 to K-231; P-155 to K-231; F-156 to K-231; D-157 to K-231; T-158 to K-231; E-159 to K-231; W-160 to K-231; Q-161 to K-231; S-162 to K-231; K-163 to K-231; Q-164 to K-231; E-165 to K-231; N-166 to K-231; T-167 to K-231; C-168 to K-231; N-169 to K-231; V-170 to K-231; T-171 to K-231; I-172 to K-231; E-173 to K-231; G-174 to K-231; L-175 to K-231; D-176 to K-231; A-177 to K-231; E-178 to K-231; K-179 to K-231; C-180 to K-231; Y-181 to K-231; S-182 to K-231; F-183 to K-231; W-184 to K-231; V-185 to K-231; R-186 to K-231; V-187 to K-231; K-188 to K-231; A-189 to K-231; M-190 to K-231; E-191 to K-231; D-192 to K-231; V-193 to K-231; Y-194 to K-231; G-



195 to K-231; P-196 to K-231; D-197 to K-231; T-198 to K-231; Y-199 to K-231; P-200 to K-231; S-201 to K-231; D-202 to K-231; W-203 to K-231; S-204 to K-231; E-205 to K-231; V-206 to K-231; T-207 to K-231; C-208 to K-231; W-209 to K-231; Q-210 to K-231; R-211 to K-231; G-212 to K-231; E-213 to K-231; I-214 to K-231; R-215 to K-231; D-216 to K-231; A-217 to K-231; C-218 to K-231; A-219 to K-231; E-220 to K-231; T-221 to K-231; P-222 to K-231; T-223 to K-231; P-224 to K-231; P-225 to K-231; K-226 to K-231; of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0119] Additionally, C-terminal deletion mutants comprising the soluble domain of SEQ ID NO:2 are also contemplated and can be described by the general formula 23- n<sup>1</sup>, where n<sup>1</sup> is an integer from 29 to 230, where n<sup>1</sup> corresponds to the position of the amino acid residue identified in SEQ ID NO:2. The invention encompasses polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of C-terminal deletions of the soluble domain of the CRCGCL polypeptide of the invention shown as SEQ ID NO:2 including polypeptides comprising the amino acid sequence of residues: Q-23 to S-230; Q-23 to L-229; Q-23 to K-228; Q-23 to P-227; Q-23 to K-226; Q-23 to P-225; Q-23 to P-224; Q-23 to T-223; Q-23 to P-222; Q-23 to T-221; Q-23 to E-220; Q-23 to A-219; Q-23 to C-218; Q-23 to A-217; Q-23 to D-216; Q-23 to R-215; Q-23 to I-214; Q-23 to E-213; Q-23 to G-212; Q-23 to R-211; Q-23 to Q-210; Q-23 to W-209; Q-23 to C-208; Q-23 to T-207; Q-23 to V-206; Q-23 to E-205; Q-23 to S-204; Q-23 to W-203; Q-23 to D-202; Q-23 to S-201; Q-23 to P-200; Q-23 to Y-199; Q-23 to T-198; Q-23 to D-197; Q-23 to P-196; Q-23 to G-195; Q-23 to Y-194; Q-23 to V-193; Q-23 to D-192; Q-23 to E-191; Q-23 to M-190; Q-23 to A-189; Q-23 to K-188; Q-23 to V-187; Q-23 to R-186; Q-23 to V-185; Q-23 to W-184; Q-23 to F-183; Q-23 to S-182; Q-23 to Y-181; Q-23 to C-180; Q-23 to K-179; Q-23 to E-178; Q-23 to A-177; Q-23 to D-176; Q-23 to L-175; Q-23 to G-174; Q-23 to E-173; Q-23 to I-172; Q-23 to T-171; Q-23 to V-170; Q-23 to N-169; Q-23 to C-168; Q-23 to T-167; Q-23 to N-166; Q-23 to E-165; Q-23 to Q-164; Q-23 to K-163; Q-23 to S-162; Q-23 to Q-161; Q-23 to W-160; Q-23 to E-159; Q-23 to T-158; Q-23 to D-157; Q-23 to F-156; Q-23 to P-155; Q-23 to S-154; Q-23 to R-153; Q-23 to Y-152; Q-23 to Q-151; Q-23 to V-150; Q-23 to E-149; Q-23 to Y-148; Q-23 to L-147; Q-23 to L-146; Q-23 to D-145; Q-23 to G-144; Q-23 to Y-143; Q-23 to S-142; Q-23 to L-141; Q-23 to D-140; Q-23 to S-139; Q-23 to C-138; Q-23 to T-137; Q-23 to V-136; Q-23 to T-135; Q-23 to V-134; Q-23 to A-133; Q-23 to D-132; Q-23 to Q-131; Q-23 to H-130; Q-23 to W-129; Q-23 to S-128; Q-23 to F-127; Q-

23 to R-126; Q-23 to V-125; Q-23 to H-124; Q-23 to K-123; Q-23 to P-122; Q-23 to S-121; Q-23 to S-120; Q-23 to P-119; Q-23 to K-118; Q-23 to L-117; Q-23 to Y-116; Q-23 to Y-115; Q-23 to V-114; Q-23 to M-113; Q-23 to W-112; Q-23 to R-111; Q-23 to S-110; Q-23 to A-109; Q-23 to T-108; Q-23 to F-107; Q-23 to V-106; Q-23 to P-105; Q-23 to H-104; Q-23 to T-103; Q-23 to G-102; Q-23 to N-101; Q-23 to R-100; Q-23 to I-99; Q-23 to S-98; Q-23 to F-97; Q-23 to Y-96; Q-23 to L-95; Q-23 to I-94; Q-23 to D-93; Q-23 to D-92; Q-23 to R-91; Q-23 to Q-90; Q-23 to E-89; Q-23 to A-88; Q-23 to D-87; Q-23 to L-86; Q-23 to L-85; Q-23 to C-84; Q-23 to G-83; Q-23 to S-82; Q-23 to T-81; Q-23 to H-80; Q-23 to G-79; Q-23 to E-78; Q-23 to Q-77; Q-23 to L-76; Q-23 to L-75; Q-23 to Y-74; Q-23 to N-73; Q-23 to T-72; Q-23 to C-71; Q-23 to Q-70; Q-23 to D-69; Q-23 to Y-68; Q-23 to A-67; Q-23 to E-66; Q-23 to D-65; Q-23 to G-64; Q-23 to N-63; Q-23 to F-62; Q-23 to R-61; Q-23 to Y-60; Q-23 to H-59; Q-23 to F-58; Q-23 to T-57; Q-23 to L-56; Q-23 to N-55; Q-23 to T-54; Q-23 to R-53; Q-23 to S-52; Q-23 to Y-51; Q-23 to K-50; Q-23 to S-49; Q-23 to A-48; Q-23 to N-47; Q-23 to W-46; Q-23 to T-45; Q-23 to V-44; Q-23 to Q-43; Q-23 to V-42; Q-23 to T-41; Q-23 to E-40; Q-23 to L-39; Q-23 to N-38; Q-23 to F-37; Q-23 to Y-36; Q-23 to I-35; Q-23 to I-34; Q-23 to Q-33; Q-23 to I-32; Q-23 to Q-31; Q-23 to V-30; Q-23 to G-29; of SEQ ID NO:2.

Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0120] Moreover, a signal sequence may be added to these C-terminal constructs. For example, amino acids 1-22 of SEQ ID NO:2, amino acids 2-22 of SEQ ID NO:2, amino acids 3-22 of SEQ ID NO:2, amino acids 4-22 of SEQ ID NO:2, amino acids 5-22 of SEQ ID NO:2, amino acids 6-22 of SEQ ID NO:2, amino acids 7-22 of SEQ ID NO:2, amino acids 8-22 of SEQ ID NO:2, amino acids 9-22 of SEQ ID NO:2, amino acids 10-22 of SEQ ID NO:2, amino acids 11-22 of SEQ ID NO:2, amino acids 12-22 of SEQ ID NO:2, amino acids 13-22 of SEQ ID NO:2, amino acids 14-22 of SEQ ID NO:2, amino acids 15-22 of SEQ ID NO:2, amino acids 16-22 of SEQ ID NO:2, amino acids 17-22 of SEQ ID NO:2, amino acids 18-22 of SEQ ID NO:2, amino acids 19-22 of SEQ ID NO:2, amino acids 20-22 of SEQ ID NO:2, or amino acids 21-22 of SEQ ID NO:2 can be added to the N-terminus of each C-terminal constructs listed above.

[0121] Also included are a nucleotide sequence encoding a polypeptide consisting of a portion of the complete CRCGCL amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209641 or 209691, where this portion excludes any integer of amino acid residues from 1 to about 361 amino acids from the amino terminus of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No.

209641 or 209691, or any integer of amino acid residues from 1 to about 361 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209641 or 209691. Polynucleotides encoding all of the above deletion mutant polypeptide forms also are provided.

[0122] The present application is also directed to proteins containing polypeptides at least 90%, 95%, 96%, 97%, 98% or 99% identical to the CRCGCL polypeptide sequence set forth herein m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific CRCGCL N- and C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0123] Among the especially preferred fragments of the invention are fragments characterized by structural or functional attributes of CRCGCL. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) of complete (i.e., full-length) CRCGCL (SEQ ID NO:2). Certain preferred regions are those set out in Figure 3 and include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence depicted in Figures 1A-1B (SEQ ID NO:2), such preferred regions include; Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Kyte-Doolittle predicted hydrophilic and hydrophobic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0124] In additional embodiments, the polynucleotides of the invention encode functional attributes of CRCGCL. Preferred embodiments of the invention in this regard include fragments that comprise, or alternatively consist of, alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions

("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of CRCGCL.

[0125] The data representing the structural or functional attributes of CRCGCL set forth in Figures 1A-1B and/or Table 1, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. In a preferred embodiment, the data presented in columns VIII, IX, XIII, and XIV of Table 1 can be used to determine regions of CRCGCL which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or IV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

[0126] Certain preferred regions in these regards are set out in Figure 3, but may, as shown in Table 1, be represented or identified by using tabular representations of the data presented in Figure 3. The DNA\*STAR computer algorithm used to generate Figure 3 (set on the original default parameters) was used to present the data in Figure 3 in a tabular format (See Table 1). The tabular format of the data in Figure 3 may be used to easily determine specific boundaries of a preferred region.

[0127] The above-mentioned preferred regions set out in Figure 3 and in Table 1 include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence set out in Figures 1A-1B. As set out in Figure 3 and in Table 1, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and coil-regions, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index.

Table 1

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met	1	.	.	B	.	.	.	.	-0.56	0.01	*	*	.	-0.10	0.59
Gly	2	.	.	B	.	.	.	.	-0.98	0.23	*	.	.	-0.10	0.34
Arg	3	.	A	B	.	.	.	.	-1.40	0.49	*	*	.	-0.60	0.22
Leu	4	.	A	B	.	.	.	.	-1.30	0.74	*	*	.	-0.60	0.18
Val	5	.	A	B	.	.	.	.	-1.26	1.04	*	*	.	-0.60	0.20
Leu	6	.	A	B	.	.	.	.	-1.24	1.04	*	*	.	-0.60	0.10
Leu	7	A	A	.	.	.	.	.	-1.49	1.54	*	*	.	-0.60	0.12
Trp	8	A	A	.	.	.	.	.	-2.46	1.36	*	*	.	-0.60	0.17
Gly	9	A	A	.	.	.	.	.	-2.34	1.36	.	.	.	-0.60	0.15
Ala	10	A	A	.	.	.	.	.	-2.30	1.46	.	.	.	-0.60	0.16
Ala	11	A	A	.	.	.	.	.	-2.30	1.46	.	.	.	-0.60	0.12
Val	12	.	A	B	.	.	.	.	-1.83	1.23	.	.	.	-0.60	0.10
Phe	13	.	A	B	.	.	.	.	-1.89	1.23	.	.	.	-0.60	0.10
Leu	14	.	A	B	.	.	.	.	-1.83	1.16	.	.	.	-0.60	0.10
Leu	15	A	.	.	.	.	T	.	-1.84	1.57	.	.	.	-0.20	0.14
Gly	16	.	.	.	.	.	T	C	-1.84	1.54	.	.	.	0.00	0.16
Gly	17	.	.	.	.	T	T	.	-1.80	1.26	.	.	.	0.20	0.19
Trp	18	A	.	.	.	.	T	.	-1.44	1.26	.	.	.	-0.20	0.19
Met	19	.	.	B	.	.	.	.	-0.63	1.00	*	.	.	-0.40	0.19
Ala	20	.	.	B	.	.	.	.	-0.17	0.97	.	.	.	-0.40	0.34
Leu	21	.	.	B	.	.	.	.	-0.17	0.97	.	.	.	-0.40	0.32
Gly	22	.	.	.	.	.	T	C	-0.41	0.49	.	.	F	0.32	0.32
Gln	23	.	.	.	.	.	T	C	-0.71	0.37	.	.	F	0.79	0.32
Gly	24	.	.	.	.	.	T	C	-0.11	0.37	.	.	F	0.96	0.39
Gly	25	.	.	.	.	.	T	C	0.13	-0.31	.	.	F	1.73	0.69
Ala	26	.	.	.	.	.	.	C	0.09	-0.31	.	.	F	1.70	0.39
Ala	27	A	.	.	.	.	.	.	0.43	-0.07	*	.	F	1.33	0.29
Glu	28	A	.	.	B	.	.	.	-0.46	-0.10	.	*	.	0.81	0.52
Gly	29	A	.	.	B	.	.	.	-0.11	0.16	.	*	.	0.04	0.36
Val	30	A	.	.	B	.	.	.	-0.66	0.06	.	*	.	-0.13	0.61
Gln	31	.	.	B	B	.	.	.	-0.96	0.24	.	*	.	-0.30	0.25
Ile	32	.	.	B	B	.	.	.	-0.61	0.93	.	.	.	-0.60	0.18
Gln	33	.	.	B	B	.	.	.	-1.31	1.26	.	.	.	-0.60	0.37
Ile	34	.	.	B	B	.	.	.	-0.97	1.40	.	*	.	-0.60	0.19
Ile	35	.	.	B	B	.	.	.	-0.92	1.40	.	*	.	-0.60	0.43
Tyr	36	.	.	B	B	.	.	.	-0.92	1.40	.	*	.	-0.60	0.20
Phe	37	.	.	B	B	.	.	.	-0.34	1.00	*	.	.	-0.60	0.50
Asn	38	.	.	.	B	.	.	C	-1.20	0.80	*	.	.	-0.25	1.03
Leu	39	.	.	.	B	.	.	C	-0.31	0.76	.	.	.	-0.40	0.49
Glu	40	.	.	B	B	.	.	.	-0.28	0.40	.	*	.	-0.60	0.98
Thr	41	.	.	B	B	.	.	.	-0.34	0.26	.	*	.	-0.30	0.45
Val	42	.	.	B	B	.	.	.	0.07	0.34	.	*	.	-0.30	0.79
Gln	43	.	.	B	B	.	.	.	0.07	0.57	.	*	.	-0.60	0.48
Val	44	.	.	B	B	.	.	.	0.29	0.97	.	*	.	-0.60	0.53
Thr	45	.	.	B	B	.	.	.	-0.01	0.99	*	*	.	-0.60	0.73
Trp	46	.	.	B	B	.	.	.	0.34	0.73	*	*	.	-0.60	0.56
Asn	47	A	.	.	B	.	.	.	0.96	0.33	*	*	.	-0.15	1.51
Ala	48	.	.	.	B	T	.	C	0.66	0.44	*	*	F	0.44	1.64
Ser	49	.	.	.	.	.	T	C	1.62	0.34	*	*	F	1.28	2.10
Lys	50	.	.	.	.	T	T	.	1.62	-0.57	*	*	F	2.72	2.55
Tyr	51	.	.	.	.	T	T	.	1.91	-0.49	*	*	F	2.76	3.65
Ser	52	.	.	.	.	T	T	.	1.10	-0.59	*	*	F	3.40	4.38
Arg	53	.	.	.	B	T	.	.	1.38	-0.29	*	*	F	2.36	1.80
Thr	54	.	.	B	B	.	.	.	0.98	0.20	*	*	F	1.02	1.66
Asn	55	.	.	B	B	.	.	.	0.90	0.23	*	*	F	0.68	1.07
Leu	56	.	.	B	B	.	.	.	0.90	0.34	*	*	.	0.04	0.75
Thr	57	.	.	B	B	.	.	.	1.31	1.10	*	*	.	-0.60	0.81
Phe	58	.	.	B	B	.	.	.	0.50	0.61	*	*	.	-0.60	0.99
His	59	.	.	B	B	.	.	.	0.81	1.00	.	*	.	-0.45	1.04

Table 1 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Tyr	60	.	.	B	B	.	.	.	0.47	0.71	.	*	.	-0.45	1.15
Arg	61	.	.	B	B	.	.	.	1.28	0.66	.	*	.	-0.45	1.32
Phe	62	.	.	.	B	T	.	.	1.59	-0.13	.	*	.	1.19	1.62
Asn	63	.	.	.	.	T	T	.	1.70	-0.63	.	*	F	2.38	1.79
Gly	64	.	.	.	.	T	T	.	1.49	-0.89	*	*	F	2.57	0.92
Asp	65	.	.	.	.	T	T	.	1.73	-0.13	.	*	F	2.76	1.67
Glu	66	.	.	.	.	T	T	.	1.62	-0.91	.	*	F	3.40	1.73
Ala	67	.	.	.	.	T	.	.	1.66	-0.91	.	.	F	2.86	3.04
Tyr	68	.	.	.	.	T	.	.	1.34	-0.77	*	.	.	2.22	0.97
Asp	69	.	.	.	.	T	.	.	1.69	-0.29	*	.	F	1.73	0.81
Gln	70	.	.	.	.	T	.	.	1.44	0.11	*	.	F	0.94	1.29
Cys	71	.	.	B	.	.	T	.	0.63	0.37	*	.	F	0.40	1.29
Thr	72	.	.	B	.	.	T	.	0.41	0.30	*	.	F	0.25	0.64
Asn	73	.	.	B	.	.	T	.	0.66	0.99	*	.	.	-0.20	0.30
Tyr	74	.	.	B	.	.	T	.	0.66	0.99	*	.	.	-0.20	0.98
Leu	75	.	.	B	.	.	.	.	0.31	0.41	*	.	.	-0.25	1.18
Leu	76	.	.	B	.	.	.	.	0.94	0.36	*	.	.	-0.10	0.73
Gln	77	.	.	B	.	.	.	.	0.94	0.46	*	.	F	0.00	0.63
Glu	78	.	.	B	.	.	.	.	0.64	0.19	*	.	F	0.70	1.10
Gly	79	.	.	.	.	T	.	.	0.54	-0.11	*	.	F	1.95	1.79
His	80	.	.	.	.	T	.	.	0.69	-0.37	.	.	F	2.20	1.02
Thr	81	.	.	.	.	T	T	.	0.69	-0.20	*	.	F	2.50	0.32
Ser	82	.	.	.	.	T	T	.	-0.12	0.49	.	.	F	1.35	0.26
Gly	83	.	.	.	.	T	T	.	-0.12	0.74	.	.	F	1.10	0.16
Cys	84	.	.	B	.	.	T	.	-0.37	0.24	.	.	.	0.60	0.19
Leu	85	.	A	B	.	.	.	.	-0.33	0.26	.	.	.	-0.05	0.14
Leu	86	A	A	.	.	.	.	.	-0.02	-0.13	*	*	.	0.30	0.24
Asp	87	A	A	.	.	.	.	.	0.39	-0.16	*	*	.	0.30	0.79
Ala	88	A	A	.	.	.	.	.	0.73	-0.73	.	*	F	0.90	1.87
Glu	89	A	A	.	.	.	.	.	1.40	-1.41	.	*	F	0.90	3.79
Gln	90	A	.	.	.	.	T	.	1.32	-2.10	.	*	F	1.30	3.79
Arg	91	A	.	.	.	.	T	.	1.32	-1.41	.	*	F	1.30	2.63
Asp	92	A	.	.	.	.	T	.	1.08	-1.23	.	*	F	1.30	1.25
Asp	93	A	.	.	.	.	T	.	0.97	-0.47	.	*	F	1.00	1.13
Ile	94	.	.	B	B	.	.	.	0.67	-0.09	.	*	.	0.30	0.50
Leu	95	.	.	B	B	.	.	.	-0.22	0.30	.	*	.	-0.30	0.40
Tyr	96	.	.	B	B	.	.	.	-0.22	0.99	.	*	.	-0.60	0.17
Phe	97	.	.	B	B	.	.	.	-0.22	0.99	*	*	.	-0.60	0.47
Ser	98	.	.	B	B	.	.	.	-0.57	0.70	*	*	.	-0.32	0.92
Ile	99	.	.	B	.	.	T	.	0.01	0.44	*	*	.	0.36	0.58
Arg	100	.	.	.	.	T	T	.	0.79	0.17	*	*	F	1.49	0.97
Asn	101	.	.	.	.	T	T	.	0.82	-0.11	*	*	F	2.37	0.98
Gly	102	.	.	.	.	T	T	.	0.67	-0.07	*	*	F	2.80	2.17
Thr	103	.	.	.	B	.	.	C	0.27	-0.11	*	*	F	1.77	0.82
His	104	.	.	.	B	.	.	C	0.84	0.67	*	*	F	0.59	0.44
Pro	105	.	.	B	B	.	.	.	0.14	0.76	*	.	.	-0.04	0.65
Val	106	.	.	B	B	.	.	.	-0.16	0.83	*	.	.	-0.32	0.45
Phe	107	.	.	B	B	.	.	.	0.30	0.73	*	.	.	-0.60	0.45
Thr	108	.	.	B	B	.	.	.	0.32	0.23	*	.	.	-0.30	0.56
Ala	109	.	.	B	B	.	.	.	-0.24	0.71	*	.	.	-0.60	0.80
Ser	110	A	.	.	B	.	.	.	-0.89	0.69	*	.	.	-0.60	0.91
Arg	111	A	.	.	B	.	.	.	-0.28	0.54	*	.	.	-0.60	0.47
Trp	112	.	.	B	B	.	.	.	0.18	0.81	*	.	.	-0.60	0.73
Met	113	.	.	B	B	.	.	.	-0.32	1.07	*	.	.	-0.60	0.85
Val	114	.	.	B	B	.	.	.	0.31	1.37	*	.	.	-0.60	0.36
Tyr	115	.	.	B	B	.	.	.	0.40	1.37	*	*	.	-0.60	0.68
Tyr	116	.	.	B	.	.	.	.	-0.01	0.89	*	*	.	-0.25	1.07
Leu	117	.	.	B	.	.	.	.	-0.02	0.66	.	.	.	0.05	1.92
Lys	118	.	.	.	.	.	T	C	0.37	0.40	.	.	F	0.90	1.65

Table 1 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Pro	119	.	.	.	.	T	T	.	1.27	0.07	.	.	F	1.70	1.62
Ser	120	.	.	.	.	.	T	C	1.48	-0.69	.	.	F	2.70	3.94
Ser	121	.	.	.	.	.	T	C	0.87	-0.87	*	*	F	3.00	2.68
Pro	122	.	.	B	.	.	.	.	1.79	-0.23	*	*	F	2.00	1.29
Lys	123	.	.	B	.	T	.	.	1.04	-0.66	*	*	F	2.40	1.88
His	124	.	.	B	.	.	.	.	0.96	-0.26	*	*	.	1.25	1.21
Val	125	.	.	B	.	.	.	.	0.97	-0.26	*	*	.	0.95	1.05
Arg	126	.	.	B	.	.	.	.	1.23	0.23	*	*	.	-0.10	0.55
Phe	127	.	.	B	.	.	.	.	1.44	0.73	*	*	.	-0.38	0.55
Ser	128	.	.	.	.	T	.	.	1.40	0.63	*	*	.	0.19	1.29
Trp	129	.	.	.	.	T	.	.	0.84	-0.01	.	*	.	1.11	1.10
His	130	.	.	.	.	.	.	C	0.84	0.49	.	*	.	0.03	1.28
Gln	131	.	.	.	B	T	.	.	0.42	0.34	.	*	.	0.20	0.71
Asp	132	.	.	.	B	T	.	.	0.27	0.44	.	.	.	-0.12	0.98
Ala	133	.	.	B	B	.	.	.	0.26	0.17	.	.	.	-0.24	0.53
Val	134	.	.	B	B	.	.	.	-0.12	0.16	.	.	.	-0.26	0.44
Thr	135	.	.	B	B	.	.	.	-0.39	0.33	.	.	.	-0.28	0.14
Val	136	.	.	B	B	.	.	.	-0.39	0.71	*	.	.	-0.60	0.19
Thr	137	.	.	B	B	.	.	.	-1.20	0.21	*	.	.	-0.30	0.43
Cys	138	.	.	B	.	.	T	.	-0.91	0.26	.	.	.	0.10	0.24
Ser	139	.	.	B	.	.	T	.	-0.30	0.16	.	.	F	0.47	0.44
Asp	140	.	.	B	.	.	T	.	-0.33	0.27	.	.	F	0.69	0.48
Leu	141	.	.	B	.	.	T	.	0.52	0.21	.	.	.	0.76	0.88
Ser	142	.	.	.	.	T	T	.	0.02	-0.36	.	.	.	2.13	1.10
Tyr	143	.	.	.	.	T	T	.	-0.12	-0.06	.	.	.	2.20	0.54
Gly	144	.	.	.	.	T	T	.	-0.07	0.63	.	.	.	1.08	0.54
Asp	145	.	.	B	.	.	T	.	-0.07	0.70	.	*	.	0.46	0.63
Leu	146	.	A	B	B	.	.	.	-0.11	0.31	*	*	.	0.14	0.70
Leu	147	.	A	B	B	.	.	.	0.19	0.20	*	*	.	-0.08	0.52
Tyr	148	.	A	B	B	.	.	.	0.19	0.17	*	*	.	-0.30	0.54
Glu	149	.	A	B	B	.	.	.	0.64	0.93	*	*	.	-0.45	1.03
Val	150	.	A	B	B	.	.	.	0.34	0.24	*	*	.	-0.15	2.45
Gln	151	.	.	B	B	.	.	.	0.94	-0.06	.	*	.	0.45	2.10
Tyr	152	.	.	.	.	T	.	.	1.06	-0.39	*	*	.	1.39	1.87
Arg	153	.	.	B	.	.	.	.	1.30	0.40	.	*	F	0.58	2.19
Ser	154	.	.	.	.	.	T	C	0.99	-0.24	.	*	F	2.22	2.11
Pro	155	.	.	.	.	.	T	C	1.84	-0.16	.	*	F	2.56	1.94
Phe	156	.	.	.	.	T	T	.	1.56	-0.91	*	*	F	3.40	1.72
Asp	157	.	.	.	.	.	T	C	1.80	0.00	*	*	F	1.96	1.35
Thr	158	.	.	.	.	.	.	C	1.39	0.01	.	*	F	1.76	1.51
Glu	159	A	.	.	.	.	.	.	1.73	-0.03	.	*	F	2.16	2.33
Trp	160	A	.	.	.	.	T	.	1.94	-0.81	.	*	F	2.66	2.80
Gln	161	A	.	.	.	.	T	.	2.64	-0.41	.	*	F	2.36	3.36
Ser	162	.	.	.	.	T	T	.	2.64	-0.90	.	*	F	3.40	3.36
Lys	163	.	.	.	.	T	T	.	2.64	-0.50	.	*	F	2.76	5.13
Gln	164	.	.	.	.	T	.	.	1.98	-0.93	.	*	F	2.69	4.28
Glu	165	.	.	.	.	T	.	.	2.27	-0.76	.	*	F	2.52	1.71
Asn	166	.	.	.	.	T	T	.	1.41	-0.74	.	*	F	2.55	1.38
Thr	167	.	.	.	.	T	T	.	1.40	-0.10	.	*	F	1.93	0.59
Cys	168	.	.	B	.	.	T	.	0.47	-0.01	.	*	F	1.70	0.49
Asn	169	.	.	B	.	.	T	.	0.47	0.67	.	*	.	0.48	0.21
Val	170	.	.	B	B	.	.	.	0.12	0.27	*	*	.	0.21	0.26
Thr	171	.	.	B	B	.	.	.	-0.69	0.21	*	*	.	0.04	0.47
Ile	172	.	A	B	B	.	.	.	-0.38	0.33	*	*	.	-0.13	0.24
Glu	173	A	A	.	B	.	.	.	-0.30	-0.07	.	*	.	0.30	0.55
Gly	174	A	A	.	.	.	.	.	-0.30	-0.21	.	*	F	0.45	0.38
Leu	175	A	A	.	.	.	.	.	0.60	-0.70	.	.	.	0.60	0.95
Asp	176	A	A	.	.	.	.	.	0.24	-1.39	.	.	F	0.90	1.09
Ala	177	A	A	.	.	.	.	.	0.89	-0.81	.	.	F	0.75	0.59
Glu	178	A	A	.	.	.	.	.	0.59	-0.49	.	.	F	0.60	1.13
Lys	179	A	A	.	B	.	.	.	0.23	-0.79	.	.	.	0.60	0.90

Table 1 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Cys	180	A	A	.	B	.	.	.	0.76	0.00	.	.	.	-0.30	0.77
Tyr	181	A	A	.	B	.	.	.	-0.10	0.41	*	*	.	-0.60	0.47
Ser	182	A	.	.	B	.	.	.	0.60	1.06	*	*	.	-0.60	0.17
Phe	183	A	.	.	B	.	.	.	-0.26	1.06	*	*	.	-0.60	0.64
Trp	184	A	.	.	B	.	.	.	-0.26	1.13	*	*	.	-0.60	0.30
Val	185	A	.	.	B	.	.	.	-0.18	0.37	.	*	.	-0.30	0.45
Arg	186	A	.	.	B	.	.	.	-0.53	0.49	.	*	.	-0.60	0.53
Val	187	A	.	.	B	.	.	.	-0.23	0.31	.	*	.	-0.30	0.49
Lys	188	A	.	.	B	.	.	.	0.47	-0.60	.	*	.	0.75	1.15
Ala	189	A	A	.	.	.	.	.	-0.10	-1.24	.	*	.	0.60	0.98
Met	190	A	A	.	.	.	.	.	0.51	-0.60	.	*	.	0.60	0.98
Glu	191	.	A	B	.	.	.	.	0.06	-0.49	.	*	.	0.30	0.77
Asp	192	.	A	B	.	.	.	.	0.70	-0.06	*	*	.	0.30	0.76
Val	193	.	A	B	.	.	.	.	0.66	-0.13	*	.	.	0.45	1.18
Tyr	194	.	A	B	.	.	.	.	0.93	-0.74	.	.	.	0.75	1.14
Gly	195	.	.	B	.	.	T	.	1.29	-0.26	.	.	F	0.85	0.98
Pro	196	.	.	.	.	T	T	.	1.08	0.50	.	.	F	0.50	2.08
Asp	197	.	.	.	.	T	T	.	0.78	0.29	.	.	F	0.80	2.05
Thr	198	.	.	.	.	.	T	C	1.63	-0.09	.	.	F	1.48	2.77
Tyr	199	.	.	B	.	.	.	C	1.59	-0.51	.	.	F	1.66	3.00
Pro	200	.	.	.	.	.	T	C	1.63	-0.03	*	.	F	2.04	1.89
Ser	201	.	.	.	.	T	T	.	1.84	0.36	*	.	F	1.92	1.75
Asp	202	.	.	.	.	T	T	.	0.99	-0.13	*	.	F	2.80	1.94
Trp	203	.	.	.	.	T	T	.	0.99	-0.24	.	.	F	2.37	0.93
Ser	204	.	.	B	B	.	.	.	0.57	-0.19	.	.	F	1.44	1.00
Glu	205	.	.	B	B	.	.	.	0.49	0.00	.	.	.	0.26	0.32
Val	206	.	.	B	B	.	.	.	0.79	0.91	*	.	.	-0.32	0.32
Thr	207	.	.	B	B	.	.	.	0.90	0.40	*	.	.	-0.60	0.42
Cys	208	.	.	.	B	T	.	.	0.84	0.01	*	*	.	0.10	0.47
Trp	209	.	.	.	.	T	T	.	1.14	0.44	.	*	.	0.20	0.63
Gln	210	A	.	.	.	.	T	.	0.26	-0.20	*	*	.	0.70	0.75
Arg	211	.	.	.	.	T	T	.	1.22	0.00	*	*	F	0.65	0.98
Gly	212	.	.	.	.	T	T	.	1.53	-0.57	*	*	F	1.70	1.83
Glu	213	.	A	.	.	T	.	.	1.61	-1.49	*	*	F	1.30	1.76
Ile	214	.	A	.	.	T	.	.	1.23	-1.39	*	*	F	1.15	0.91
Arg	215	.	A	.	.	T	.	.	0.64	-0.81	*	*	F	1.15	0.49
Asp	216	.	A	.	.	T	.	.	0.53	-0.74	*	*	F	1.15	0.29
Ala	217	.	A	.	.	T	.	.	0.57	-0.74	*	*	.	1.00	0.71
Cys	218	.	A	B	.	.	.	.	0.36	-0.94	*	*	.	0.60	0.52
Ala	219	.	A	.	.	T	.	.	0.93	-0.51	*	.	.	1.00	0.48
Glu	220	.	A	.	.	T	.	.	0.61	-0.03	.	.	F	0.85	0.69
Thr	221	.	A	.	.	.	.	C	0.40	-0.10	.	*	F	1.14	2.00
Pro	222	.	A	.	.	T	.	.	1.03	-0.24	.	.	F	1.68	3.06
Thr	223	.	.	.	.	.	.	C	1.49	-0.74	.	*	F	2.32	3.53
Pro	224	.	.	.	.	.	T	C	2.12	-0.31	.	*	F	2.56	3.78
Pro	225	.	.	.	.	T	T	.	1.31	-0.80	.	*	F	3.40	4.89
Lys	226	.	.	.	.	.	T	C	1.32	-0.54	.	*	F	2.86	2.80
Pro	227	A	.	.	.	.	T	.	1.58	-0.64	*	.	F	2.32	2.42
Lys	228	A	.	.	.	.	.	.	1.19	-1.07	*	*	F	1.78	3.13
Leu	229	A	.	.	B	.	.	.	0.51	-0.71	*	*	F	1.24	1.36
Ser	230	.	.	B	B	.	.	.	-0.09	-0.03	.	*	F	0.45	0.62
Lys	231	.	.	B	B	.	.	.	-1.02	0.23	.	*	.	-0.30	0.25
Phe	232	.	.	B	B	.	.	.	-1.11	0.91	*	.	.	-0.60	0.22
Ile	233	.	.	B	B	.	.	.	-1.46	0.61	.	.	.	-0.60	0.22
Leu	234	.	.	B	B	.	.	.	-1.46	0.61	.	.	.	-0.60	0.14
Ile	235	.	.	B	B	.	.	.	-1.74	1.30	.	.	.	-0.60	0.14
Ser	236	A	.	.	B	.	.	.	-2.68	1.01	.	.	.	-0.60	0.20
Ser	237	A	.	.	B	.	.	.	-2.79	1.01	.	.	.	-0.60	0.17
Leu	238	A	.	.	B	.	.	.	-2.71	1.01	.	.	.	-0.60	0.20
Ala	239	A	.	.	B	.	.	.	-2.50	1.01	.	.	.	-0.60	0.12
Ile	240	A	.	.	B	.	.	.	-2.47	1.24	.	.	.	-0.60	0.09



Table 1 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Leu	241	A	.	.	B	.	.	.	-2.47	1.50	.	*	.	-0.60	0.08
Leu	242	A	.	.	B	.	.	.	-2.98	1.20	.	.	.	-0.60	0.11
Met	243	.	.	B	B	.	.	.	-2.98	1.39	.	*	.	-0.60	0.13
Val	244	A	.	.	B	.	.	.	-3.20	1.39	.	.	.	-0.60	0.13
Ser	245	A	.	.	B	.	.	.	-3.12	1.39	.	.	.	-0.60	0.13
Leu	246	A	.	.	B	.	.	.	-2.61	1.39	.	.	.	-0.60	0.11
Leu	247	A	.	.	B	.	.	.	-2.61	1.16	.	.	.	-0.60	0.19
Leu	248	A	.	.	B	.	.	.	-2.30	1.20	*	.	.	-0.60	0.12
Leu	249	A	.	.	B	.	.	.	-1.40	1.73	*	.	.	-0.60	0.15
Ser	250	A	.	.	B	.	.	.	-1.91	1.04	*	.	.	-0.60	0.36
Leu	251	A	.	.	B	.	.	.	-1.39	1.04	*	.	.	-0.60	0.36
Trp	252	A	.	.	B	.	.	.	-0.47	1.27	*	.	.	-0.60	0.46
Lys	253	A	.	.	B	.	.	.	-0.51	0.59	*	*	.	-0.60	0.68
Leu	254	A	.	.	B	.	.	.	0.34	0.84	*	*	.	-0.60	0.61
Trp	255	A	.	.	B	.	.	.	0.69	0.16	*	.	.	-0.15	1.16
Arg	256	A	.	.	B	.	.	.	0.80	-0.76	*	.	.	0.75	1.16
Val	257	A	.	.	B	.	.	.	0.28	0.03	*	.	.	-0.15	1.21
Lys	258	.	.	B	B	.	.	.	-0.66	0.03	*	.	.	-0.30	0.95
Lys	259	.	.	B	B	.	.	.	-0.06	-0.20	*	.	.	0.30	0.34
Phe	260	.	.	B	B	.	.	.	-0.07	0.23	*	*	.	-0.30	0.71
Leu	261	.	.	B	B	.	.	.	-1.03	-0.03	*	*	.	0.30	0.48
Ile	262	.	.	B	B	.	.	.	-0.39	0.61	*	.	.	-0.60	0.18
Pro	263	.	.	B	.	.	.	.	-0.43	1.04	*	.	.	-0.10	0.32
Ser	264	.	.	B	.	.	.	.	-0.69	0.26	*	*	F	0.65	0.64
Val	265	.	.	.	.	.	.	C	0.06	0.00	.	.	F	1.30	1.41
Pro	266	.	.	.	.	.	.	C	0.57	-0.69	.	.	F	2.50	1.82
Asp	267	.	.	.	.	.	T	C	0.57	-0.73	.	.	F	3.00	1.82
Pro	268	.	.	.	.	T	T	.	0.08	-0.43	.	.	F	2.60	1.72
Lys	269	.	.	.	.	T	T	.	0.17	-0.29	.	.	F	2.15	0.96
Ser	270	.	.	B	.	.	T	.	0.68	-0.29	*	.	F	1.45	0.89
Ile	271	.	.	B	.	.	.	.	0.08	0.14	*	.	.	0.20	0.57
Phe	272	.	.	B	.	.	T	.	-0.62	0.40	*	.	.	-0.20	0.24
Pro	273	.	.	B	.	.	T	.	-0.41	1.19	*	*	.	-0.20	0.15
Gly	274	.	.	.	.	.	T	C	-1.34	0.80	*	*	.	0.00	0.38
Leu	275	.	.	B	.	.	T	.	-1.08	0.80	*	.	.	-0.20	0.30
Phe	276	.	.	B	.	.	.	.	-0.19	0.51	*	.	.	-0.40	0.27
Glu	277	A	.	.	.	.	.	.	0.17	0.49	.	*	.	-0.40	0.47
Ile	278	A	.	.	.	.	.	.	0.38	0.49	.	.	.	-0.40	0.56
His	279	A	.	.	.	.	T	.	0.02	0.20	*	*	.	0.25	1.04
Gln	280	.	.	.	.	.	T	C	0.83	0.20	*	.	F	0.45	0.52
Gly	281	.	.	.	.	.	T	C	1.53	0.60	*	.	F	0.30	1.29
Asn	282	.	.	.	.	.	T	C	1.24	-0.09	.	.	F	1.20	1.64
Phe	283	.	A	.	.	.	.	C	1.24	0.33	.	.	F	0.05	1.00
Gln	284	.	A	.	.	.	.	C	0.97	0.61	*	*	.	-0.16	0.71
Glu	285	.	A	B	.	.	.	.	0.97	0.67	*	*	.	-0.12	0.63
Trp	286	.	A	B	.	.	.	.	1.00	0.27	*	*	.	0.57	1.22
Ile	287	.	A	B	.	.	.	.	1.00	-0.03	*	.	.	1.41	1.02
Thr	288	.	.	.	.	T	.	.	1.70	-0.03	*	.	F	2.40	1.02
Asp	289	.	.	.	.	T	.	.	0.84	0.37	*	.	F	1.56	1.56
Thr	290	A	.	.	.	.	.	.	0.26	0.10	*	.	F	0.92	1.65
Gln	291	A	A	.	.	.	.	.	0.51	-0.09	*	.	F	1.08	1.15
Asn	292	A	A	.	.	.	.	.	0.59	-0.07	.	.	F	0.69	0.94
Val	293	A	A	.	.	.	.	.	0.87	0.61	*	.	.	-0.60	0.54
Ala	294	A	A	.	.	.	.	.	0.91	0.63	*	.	.	-0.60	0.42
His	295	A	A	.	.	.	.	.	0.62	0.23	*	.	.	-0.30	0.52
Leu	296	A	A	.	.	.	.	.	0.03	0.44	*	.	.	-0.60	0.70
His	297	A	A	.	.	.	.	.	-0.31	0.30	*	.	.	-0.30	0.70
Lys	298	A	A	.	.	.	.	.	-0.04	0.23	*	.	.	-0.30	0.51
Met	299	A	A	.	.	.	.	.	0.54	0.23	.	.	.	-0.30	0.62
Ala	300	A	A	.	.	.	.	.	0.58	-0.46	*	.	.	0.30	0.79
Gly	301	A	A	.	.	.	.	.	1.39	-0.56	*	.	.	0.60	0.69

Table 1 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Ala	302	A	A	.	.	.	.	.	1.12	-0.56	.	.	F	0.90	1.20
Glu	303	A	A	.	.	.	.	.	0.73	-0.79	.	.	F	1.20	1.60
Gln	304	A	A	.	.	.	.	.	1.12	-0.86	.	.	F	1.50	1.60
Glu	305	.	A	.	.	.	.	C	1.71	-0.86	.	.	F	2.00	2.44
Ser	306	.	A	.	.	.	.	C	2.06	-1.36	.	.	F	2.30	2.44
Gly	307	.	.	.	.	.	T	C	2.43	-1.36	.	.	F	3.00	2.44
Pro	308	.	.	.	.	.	T	C	1.62	-1.33	.	.	F	2.70	2.18
Glu	309	.	.	.	.	.	T	C	0.77	-0.64	.	.	F	2.40	1.34
Glu	310	A	.	.	.	.	T	.	-0.09	-0.39	.	.	F	1.60	1.01
Pro	311	A	.	.	.	.	.	.	0.21	-0.17	.	.	F	0.95	0.48
Leu	312	A	A	.	.	.	.	.	-0.26	-0.20	.	.	.	0.30	0.48
Val	313	A	A	.	.	.	.	.	-0.63	0.49	*	.	.	-0.60	0.23
Val	314	A	A	.	.	.	.	.	-0.59	0.99	*	.	.	-0.60	0.15
Gln	315	A	A	.	.	.	.	.	-0.90	0.56	*	.	.	-0.60	0.36
Leu	316	A	A	.	.	.	.	.	-0.69	0.36	*	.	.	-0.30	0.71
Ala	317	A	A	.	.	.	.	.	-0.47	-0.29	*	*	.	0.45	1.65
Lys	318	A	A	.	.	.	.	.	0.39	-0.43	*	.	F	0.45	0.96
Thr	319	A	A	.	.	.	.	.	0.94	-0.83	*	.	F	0.90	2.03
Glu	320	A	A	.	.	.	.	.	0.73	-1.13	*	.	F	0.90	2.69
Ala	321	A	A	.	.	.	.	.	1.66	-1.20	*	.	F	1.24	2.08
Glu	322	A	A	.	.	.	.	.	1.64	-1.20	*	*	F	1.58	2.82
Ser	323	A	.	.	.	.	T	.	0.79	-1.07	*	.	F	2.32	1.61
Pro	324	A	.	.	.	.	T	.	1.10	-0.39	*	.	F	2.36	1.32
Arg	325	.	.	.	.	T	T	.	0.89	-0.89	*	.	F	3.40	1.27
Met	326	A	.	.	.	.	T	.	1.48	-0.46	*	.	.	2.21	1.46
Leu	327	A	.	.	.	.	.	.	1.17	-0.44	*	.	F	1.82	1.64
Asp	328	A	.	.	.	.	T	.	1.47	-0.39	*	.	F	1.68	1.21
Pro	329	A	.	.	.	.	T	.	1.68	-0.39	*	*	F	1.34	2.11
Gln	330	A	.	.	.	.	T	.	1.61	-1.00	*	.	F	1.30	4.44
Thr	331	A	.	.	.	.	T	.	2.21	-1.69	.	*	F	1.30	5.31
Glu	332	A	A	.	.	.	.	.	2.43	-1.69	.	*	F	0.90	5.95
Glu	333	A	A	.	.	.	.	.	2.13	-1.61	.	.	F	0.90	3.47
Lys	334	A	A	.	.	.	.	.	2.00	-1.63	.	.	F	1.15	3.22
Glu	335	A	A	.	.	.	.	.	1.66	-1.69	.	.	F	1.40	1.84
Ala	336	A	.	.	.	.	T	.	1.67	-1.26	.	.	F	2.05	1.05
Ser	337	A	.	.	.	.	T	.	0.86	-0.87	.	.	F	2.15	0.71
Gly	338	.	.	.	.	T	T	.	0.86	-0.19	.	*	F	2.50	0.34
Gly	339	.	.	.	.	T	T	.	-0.00	0.21	*	.	F	1.65	0.58
Ser	340	.	.	.	.	.	.	C	-0.21	0.40	.	.	F	0.70	0.35
Leu	341	.	.	.	.	.	.	C	0.34	0.44	.	*	.	0.30	0.55
Gln	342	.	.	B	.	.	.	.	0.64	0.51	.	*	.	-0.15	0.76
Leu	343	.	.	B	.	.	.	.	0.78	0.49	.	*	.	-0.40	0.98
Pro	344	.	.	B	.	.	.	.	0.31	0.53	*	*	.	-0.25	1.84
His	345	.	.	B	.	.	.	.	0.61	0.53	*	*	.	-0.40	0.88
Gln	346	.	.	B	.	.	.	.	1.08	0.53	*	.	F	0.03	1.84
Pro	347	.	.	B	.	.	.	.	0.73	0.27	*	.	F	0.46	1.18
Leu	348	.	.	.	.	T	T	.	1.54	0.27	*	.	F	1.04	0.86
Gln	349	.	.	.	.	T	T	.	0.90	-0.23	*	.	F	1.77	0.83
Gly	350	.	.	.	.	T	T	.	0.08	0.01	*	.	F	1.30	0.40
Gly	351	.	.	B	.	.	T	.	-0.23	0.23	.	.	F	0.77	0.36
Asp	352	.	.	B	B	.	.	.	-0.91	0.03	.	.	F	0.24	0.30
Val	353	.	.	B	B	.	.	.	-0.44	0.31	.	.	F	0.11	0.21
Val	354	.	.	B	B	.	.	.	-0.79	0.31	.	.	.	-0.17	0.21
Thr	355	.	.	B	B	.	.	.	-1.14	0.31	.	.	.	-0.30	0.13
Ile	356	.	.	B	B	.	.	.	-1.11	1.10	.	.	.	-0.60	0.15
Gly	357	.	.	B	B	.	.	.	-1.81	0.94	.	.	.	-0.60	0.28
Gly	358	.	.	B	B	.	.	.	-1.81	1.09	.	.	.	-0.60	0.17
Phe	359	.	.	B	B	.	.	.	-1.56	1.24	.	.	.	-0.60	0.18
Thr	360	.	.	B	B	.	.	.	-1.24	1.17	.	.	.	-0.60	0.18
Phe	361	.	.	B	B	.	.	.	-0.36	1.14	.	*	.	-0.60	0.29
Val	362	.	.	B	B	.	.	.	0.10	0.71	.	*	.	-0.32	0.57

**Table 1 (continued)**

Res Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met 363	.	.	B	B	.	.	.	0.14	-0.07	.	*	.	0.86	0.77
Asn 364	.	.	.	B	T	.	.	0.60	-0.17	.	*	.	1.69	1.19
Asp 365	.	.	.	.	T	T	.	0.06	-0.20	.	*	.	2.37	2.51
Arg 366	.	.	.	.	T	T	.	0.17	-0.20	.	*	F	2.80	1.88
Ser 367	A	.	.	.	.	T	.	0.21	-0.31	.	*	.	1.97	1.18
Tyr 368	A	.	.	.	.	T	.	0.42	-0.03	.	*	.	1.54	0.58
Val 369	.	A	B	.	.	.	.	0.03	0.40	.	*	.	-0.04	0.38
Ala 370	.	A	B	.	.	.	.	-0.36	0.83	*	.	.	-0.32	0.36
Leu 371	.	A	B	.	.	.	.	-0.86	0.87	*	*	.	-0.60	0.30

[0128] Among highly preferred fragments in this regard are those that comprise regions of CRCGCL that combine several structural features, such as several of the features set out above.

[0129] Other preferred fragments are biologically active CRCGCL fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the CRCGCL polypeptide. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

[0130] However, many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:1 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. One embodiment of the present invention excludes Genbank Accession No. X91553 (herein incorporated by reference in its entirety.) Moreover, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1559 of SEQ ID NO:1, b is an integer of 15 to 1573, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:1, and where the b is greater than or equal to a + 14.

#### **Epitopes and Antibodies**

[0131] The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:2, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. 209691 or 209641 or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:1 or contained in ATCC deposit No. 209691 or 209641 under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:2), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent

hybridization conditions or alternatively under lower stringency hybridization conditions defined supra.

[0132] The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic. Either the full-length protein or an antigenic peptide fragment can be used. Regions having a high antigenicity index are shown in Table 1 and Figure 3.

[0133] Antibodies are preferably prepared from these regions or from discrete fragments in these regions. However, antibodies can be prepared from any region of the peptide as described herein. A preferred fragment produces an antibody that diminishes or completely prevents binding of CRCGCL to its ligand (e.g., TSLP). Antibodies can be developed against the full length CRCGCL or portions of CRCGCL, for example, the mature form of CRCGCL polypeptide, the extracellular soluble form of CRCGCL, or any portions of these regions. Antibodies may also be developed against specific functional sites, such as the site of ligand binding or sites that are glycosylated, phosphorylated, myristoylated, or amidated.

[0134] Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

[0135] In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at

least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., *Cell* 37:767-778 (1984); Sutcliffe et al., *Science* 219:660-666 (1983)). These fragments are not to be construed, however, as encompassing any fragments which may be disclosed prior to the invention.

[0136] Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle et al., *J. Gen. Virol.* 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

[0137] Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-

hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

[0138] Epitope bearing peptides of the invention may also be synthesized as multiple antigen peptides (MAPs), first described by J. P. Tam in *Proc. Natl. Acad. Sci. U.S.A.* 85:5409, which is incorporated by reference herein in its entirety. MAPs consist of multiple copies of a specific peptide attached to a non-immunogenic lysine core. MAP peptides usually contain four or eight copies of the peptide often referred to as MAP-4 or MAP-8 peptides. By way of non-limiting example, MAPs may be synthesized onto a lysine core matrix attached to a polyethylene glycol-polystyrene (PEG-PS) support. The peptide of choice is synthesized onto the lysine residues using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry. For example, Applied Biosystems (Foster City, CA) offers MAP resins, such as, for example, the Fmoc Resin 4 Branch and the Fmoc Resin 8 Branch which can be used to synthesize MAPs. Cleavage of MAPs from the resin is performed with standard trifluoroacetic acid (TFA)-based cocktails known in the art. Purification of MAPs, except for desalting, is not necessary. MAP peptides may be used as an immunizing vaccine which elicits antibodies that recognize both the MAP and the native protein from which the peptide was derived.

[0139] Epitope bearing polypeptides of the invention may be modified, for example, by the addition of amino acids at the amino- and/or carboxy- termini of the peptide. Such modifications may be performed, for example, to alter the conformation of the epitope bearing polypeptide such that the epitope will have a conformation more closely related to the structure of the epitope in the native protein. An example of a modified epitope-bearing polypeptide of the invention is a polypeptide in which one or more cysteine residues have been added to the polypeptide to allow for the formation of a disulfide bond between two

cysteines, resulting in a stable loop structure of the epitope bearing polypeptide under non-reducing conditions. Disulfide bonds may form between a cysteine residue added to the polypeptide and a cysteine residue of the naturally occurring epitope, or may form between two cysteines which have both been added to the naturally occurring epitope bearing polypeptide. Additionally, it is possible to modify one or more amino acid residues of the naturally occurring epitope bearing polypeptide by substituting them with cysteines to promote the formation of disulfide bonded loop structures. Cyclic thioether molecules of synthetic peptides may be routinely generated using techniques known in the art and are described in PCT publication WO 97/46251, incorporated in its entirety by reference herein. Other modifications of epitope-bearing polypeptides contemplated by this invention include biotinylation.

[0140] Animals such as rabbits, rats and mice are immunized with either free or carrier-coupled peptides or MAP peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

[0141] As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention (e.g., those comprising an immunogenic or antigenic epitope) can be fused to other heterologous polypeptide sequences. For example, the polypeptides of the present invention (including fragments or variants thereof), may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. By way of another non-limiting example, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused with albumin (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their



entirety)). In a preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. Polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide). Polynucleotides encoding fusion proteins of the invention are also encompassed by the invention.

[0142] Such fusion proteins as those described above may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein.

Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni<sup>2+</sup>-nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

[0143] Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., *Curr. Opin. Biotechnol.* 8:724-33 (1997); Harayama, *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo and Blasco, *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:1 and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

### Antibodies

[0144] Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:2, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding).

[0145] The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion

of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. *See generally, Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site.

[0146] Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

[0147] The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the heavy and the light chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J Mol. Biol.* 196:901-917 (1987); Chothia et al. *Nature* 342:878-883 (1989).

[0148] A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. *See, e.g.,* Songsivilai & Lachmann *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny et al. *J Immunol.* 148:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" (Holliger et al. "Diabodies: small bivalent and bispecific antibody fragments" *PNAS USA* 90:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)).

[0149] Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression

library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In a preferred embodiment, the immunoglobulin is an IgG1 isotype. In another preferred embodiment, the immunoglobulin is an IgG2 isotype. In another preferred embodiment, the immunoglobulin is an IgG4 isotype. Immunoglobulins may have both a heavy and light chain. An array of IgG, IgE, IgM, IgD, IgA, and IgY heavy chains may be paired with a light chain of the kappa or lambda forms.

[0150] Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

[0151] The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69

(1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

[0152] Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

[0153] Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, monkey, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein).

[0154] In preferred embodiments, the antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), immunospecifically bind to CRCGCL and do not cross-react with any other antigens. In preferred embodiments, the antibodies of the invention immunospecifically bind to CRCGCL

and do not cross-react with other type I cytokine receptor chains such as, for example, IL-2R gamma chain, IL-2R alpha chain, IL-2R beta chain, IL-3R alpha chain, IL-3R beta chain, IL-4R alpha chain, IL-5R alpha chain, IL-6R alpha chain, IL-6R beta chain, IL-7R alpha chain, IL-9R chain, IL-11R alpha chain, IL-12R alpha chain, IL-13R alpha chain, and/or IL-15R chain.

[0155] In other preferred embodiments, the antibodies of the invention immunspecifically bind to CRGCL and cross-react with other type I cytokine receptor chains such as, for example, IL-2R gamma chain, IL-2R alpha chain, IL-2R beta chain, IL-3R alpha chain, IL-3R beta chain, IL-4R alpha chain, IL-5R alpha chain, IL-6R alpha chain, IL-6R beta chain, IL-7R alpha chain, IL-9R chain, IL-11R alpha chain, IL-12R alpha chain, IL-13R alpha chain, and/or IL-15R chain. In a preferred embodiment, antibodies of the invention preferentially bind CRGCL (SEQ ID NO:2), or fragments and variants thereof relative to their ability to bind other antigens, (such as, for example, other type I cytokine receptor chains).

[0156] By way of non-limiting example, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with a dissociation constant ( $K_D$ ) that is less than the antibody's  $K_D$  for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an affinity that is at least one order of magnitude less than the antibody's  $K_D$  for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an affinity that is at least two orders of magnitude less than the antibody's  $K_D$  for the second antigen.

[0157] In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an off rate ( $k_{off}$ ) that is less than the antibody's  $k_{off}$  for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an affinity that is at least one order of magnitude less than the antibody's  $k_{off}$  for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an affinity that is at least two orders of magnitude less than the antibody's  $k_{off}$  for the second antigen.

[0158] Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities

include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2} \text{ M}$ ,  $10^{-2} \text{ M}$ ,  $5 \times 10^{-3} \text{ M}$ ,  $10^{-3} \text{ M}$ ,  $5 \times 10^{-4} \text{ M}$ , or  $10^{-4} \text{ M}$ . More preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-5} \text{ M}$ ,  $10^{-5} \text{ M}$ ,  $5 \times 10^{-6} \text{ M}$ ,  $10^{-6} \text{ M}$ ,  $5 \times 10^{-7} \text{ M}$ ,  $10^{-7} \text{ M}$ ,  $5 \times 10^{-8} \text{ M}$  or  $10^{-8} \text{ M}$ . Even more preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-9} \text{ M}$ ,  $10^{-9} \text{ M}$ ,  $5 \times 10^{-10} \text{ M}$ ,  $10^{-10} \text{ M}$ ,  $5 \times 10^{-11} \text{ M}$ ,  $10^{-11} \text{ M}$ ,  $5 \times 10^{-12} \text{ M}$ ,  $10^{-12} \text{ M}$ ,  $5 \times 10^{-13} \text{ M}$ ,  $10^{-13} \text{ M}$ ,  $5 \times 10^{-14} \text{ M}$ ,  $10^{-14} \text{ M}$ ,  $5 \times 10^{-15} \text{ M}$ , or  $10^{-15} \text{ M}$ .

[0159] In specific embodiments, antibodies of the invention bind CRCGCL polypeptides or fragments or variants thereof with an off rate ( $k_{\text{off}}$ ) of less than or equal to  $5 \times 10^{-2} \text{ sec}^{-1}$ ,  $10^{-2} \text{ sec}^{-1}$ ,  $5 \times 10^{-3} \text{ sec}^{-1}$  or  $10^{-3} \text{ sec}^{-1}$ . More preferably, antibodies of the invention bind CRCGCL polypeptides or fragments or variants thereof with an off rate ( $k_{\text{off}}$ ) less than or equal to  $5 \times 10^{-4} \text{ sec}^{-1}$ ,  $10^{-4} \text{ sec}^{-1}$ ,  $5 \times 10^{-5} \text{ sec}^{-1}$ , or  $10^{-5} \text{ sec}^{-1}$ ,  $5 \times 10^{-6} \text{ sec}^{-1}$ ,  $10^{-6} \text{ sec}^{-1}$ ,  $5 \times 10^{-7} \text{ sec}^{-1}$  or  $10^{-7} \text{ sec}^{-1}$ .

[0160] In other embodiments, antibodies of the invention bind CRCGCL polypeptides or fragments or variants thereof with an on rate ( $k_{\text{on}}$ ) of greater than or equal to  $10^3 \text{ M}^{-1} \text{ sec}^{-1}$ ,  $5 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$ ,  $10^4 \text{ M}^{-1} \text{ sec}^{-1}$  or  $5 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$ . More preferably, antibodies of the invention bind CRCGCL polypeptides or fragments or variants thereof with an on rate ( $k_{\text{on}}$ ) greater than or equal to  $10^5 \text{ M}^{-1} \text{ sec}^{-1}$ ,  $5 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}$ ,  $10^6 \text{ M}^{-1} \text{ sec}^{-1}$ , or  $5 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$  or  $10^7 \text{ M}^{-1} \text{ sec}^{-1}$ .

[0161] The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

[0162] Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation

(i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

[0163] The invention also features CRCGCL-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the CRCGCL-ligand complex (e.g., CRCGCL bound to TSLP), and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand (e.g., TSLP) and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing CRCGCL activation, but do not prevent the ligand from binding the CRCGCL. The invention also features CRCGCL-specific antibodies that recognize the CRCGCL-type I cytokine receptor chain complex (including, but not limited to, CRCGCL complexed with IL-7R alpha chain), and, preferably, do not specifically recognize the unbound receptor chains. Further included in the invention are antibodies which activate CRCGCL. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of CRCGCL or inducing heterodimerization (e.g., the dimerization of CRCGCL with a heterologous polypeptide, such as, for example, the IL-7 receptor alpha chain). The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek



et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

[0164] Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

[0165] As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

[0166] The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0167] The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen.

Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

[0168] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0169] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 16). In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[0170] Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the

invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

[0171] Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

[0172] For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0173] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria,

e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

[0174] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol. Methods* 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991);

Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

[0175] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[0176] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent

No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0177] Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

[0178] Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, *J. Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

#### *Polynucleotides Encoding Antibodies*

[0179] The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:2.

[0180] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide

sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0181] Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A<sup>+</sup> RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[0182] Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0183] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the

regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

[0184] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

[0185] Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423-42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., Science 242:1038-1041 (1988)).

#### *Methods of Producing Antibodies*



[0186] The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

[0187] Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

[0188] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0189] A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

[0190] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol.*

Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0191] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0192] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

[0193] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure

the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

[0194] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

[0195] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgp<sup>r</sup>t- or ap<sup>r</sup>t- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem.

62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygromycin resistance (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

[0196] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

[0197] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0198] Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of

the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

[0199] The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

[0200] The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et

al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337-11341(1992) (said references incorporated by reference in their entireties).

[0201] As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:2 may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:2 may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

[0202] Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

[0203] The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{111}\text{In}$  or  $^{99}\text{Tc}$ .

[0204] Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and



cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0205] The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[0206] Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[0207] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*,

"The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

[0208] In specific embodiments, CRCGCL binding polypeptides of the invention are attached either directly or indirectly, to macrocyclic chelators useful for chelating radiometal ions, including but not limited to  $^{177}\text{Lu}$ ,  $^{90}\text{Y}$ ,  $^{166}\text{Ho}$ , and  $^{153}\text{Sm}$ , to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators attached to CRCGCL polypeptides of the invention is  $^{111}\text{In}$ . In another preferred embodiment, the radiometal ion associated with the macrocyclic chelator attached to CRCGCL polypeptides of the invention is  $^{90}\text{Y}$ . In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane- $\text{N,N',N'',N'''}\text{-tetraacetic acid}$  (DOTA). In one embodiment the side chain moiety of one or more classical or non-classical amino acids in a CRCGCL binding polypeptide comprises a DOTA molecule. In other specific embodiments, the DOTA is attached to the CRCGCL binding polypeptide of the invention via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., *Clin. Cancer Res.*, 4(10):2483-90 (1998); Peterson et al., *Bioconjug. Chem.*, 10(4):553-7 (1999); and Zimmerman et al, *Nucl. Med. Biol.*, 26(8):943-50 (1999), which are hereby incorporated by reference in their entirety. In addition, U.S. Patents 5,652,361 and 5,756,065, which disclose chelating agents that may be conjugated to antibodies, and methods for making and using them, are hereby incorporated by reference in their entirety. Though U.S. Patents 5,652,361 and 5,756,065 focus on conjugating chelating agents to antibodies, one skilled in the art could readily adapt the methods disclosed therein in order to conjugate chelating agents to other polypeptides.

[0209] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

[0210] An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

### *Immunophenotyping*

[0211] The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention

may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

[0212] These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

#### *Assays For Antibody Binding*

[0213] The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

[0214] Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasyolol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time

(e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

[0215] Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., 32P or 125I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

[0216] ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to

a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

[0217] The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second antibody.

#### *Therapeutic Uses*

[0218] The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating, detecting, diagnosing and/or preventing one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a CRCGCL polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be

provided in pharmaceutically acceptable compositions as known in the art or as described herein.

[0219] A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

[0220] The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

[0221] The antibodies of the invention may be administered alone or in combination with other monoclonals or chimeric antibodies directed to CCRGCL ligands or fragments thereof (e.g., TSLP), and/or other type I cytokine receptor chains (e.g., IL-7 receptor alpha chain)

[0222] The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

[0223] It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M,  $10^{-15}$  M,  $5 \times 10^{-16}$  M,  $10^{-16}$  M,  $5 \times 10^{-17}$  M,  $10^{-17}$  M,  $5 \times 10^{-18}$  M,  $10^{-18}$  M,  $5 \times 10^{-19}$  M,  $10^{-19}$  M,  $5 \times 10^{-20}$  M,  $10^{-20}$  M,  $5 \times 10^{-21}$  M,  $10^{-21}$  M,  $5 \times 10^{-22}$  M,  $10^{-22}$  M,  $5 \times 10^{-23}$  M,  $10^{-23}$  M,  $5 \times 10^{-24}$  M,  $10^{-24}$  M,  $5 \times 10^{-25}$  M,  $10^{-25}$  M,  $5 \times 10^{-26}$  M,  $10^{-26}$  M,  $5 \times 10^{-27}$  M,  $10^{-27}$  M,  $5 \times 10^{-28}$  M,  $10^{-28}$  M,  $5 \times 10^{-29}$  M,  $10^{-29}$  M,  $5 \times 10^{-30}$  M,  $10^{-30}$  M,  $5 \times 10^{-31}$  M,  $10^{-31}$  M,  $5 \times 10^{-32}$  M,  $10^{-32}$  M,  $5 \times 10^{-33}$  M,  $10^{-33}$  M,  $5 \times 10^{-34}$  M,  $10^{-34}$  M,  $5 \times 10^{-35}$  M,  $10^{-35}$  M,  $5 \times 10^{-36}$  M,  $10^{-36}$  M,  $5 \times 10^{-37}$  M,  $10^{-37}$  M,  $5 \times 10^{-38}$  M,  $10^{-38}$  M,  $5 \times 10^{-39}$  M,  $10^{-39}$  M,  $5 \times 10^{-40}$  M,  $10^{-40}$  M,  $5 \times 10^{-41}$  M,  $10^{-41}$  M,  $5 \times 10^{-42}$  M,  $10^{-42}$  M,  $5 \times 10^{-43}$  M,  $10^{-43}$  M,  $5 \times 10^{-44}$  M,  $10^{-44}$  M,  $5 \times 10^{-45}$  M,  $10^{-45}$  M,  $5 \times 10^{-46}$  M,  $10^{-46}$  M,  $5 \times 10^{-47}$  M,  $10^{-47}$  M,  $5 \times 10^{-48}$  M,  $10^{-48}$  M,  $5 \times 10^{-49}$  M,  $10^{-49}$  M,  $5 \times 10^{-50}$  M,  $10^{-50}$  M,  $5 \times 10^{-51}$  M,  $10^{-51}$  M,  $5 \times 10^{-52}$  M,  $10^{-52}$  M,  $5 \times 10^{-53}$  M,  $10^{-53}$  M,  $5 \times 10^{-54}$  M,  $10^{-54}$  M,  $5 \times 10^{-55}$  M,  $10^{-55}$  M,  $5 \times 10^{-56}$  M,  $10^{-56}$  M,  $5 \times 10^{-57}$  M,  $10^{-57}$  M,  $5 \times 10^{-58}$  M,  $10^{-58}$  M,  $5 \times 10^{-59}$  M,  $10^{-59}$  M,  $5 \times 10^{-60}$  M,  $10^{-60}$  M,  $5 \times 10^{-61}$  M,  $10^{-61}$  M,  $5 \times 10^{-62}$  M,  $10^{-62}$  M,  $5 \times 10^{-63}$  M,  $10^{-63}$  M,  $5 \times 10^{-64}$  M,  $10^{-64}$  M,  $5 \times 10^{-65}$  M,  $10^{-65}$  M,  $5 \times 10^{-66}$  M,  $10^{-66}$  M,  $5 \times 10^{-67}$  M,  $10^{-67}$  M,  $5 \times 10^{-68}$  M,  $10^{-68}$  M,  $5 \times 10^{-69}$  M,  $10^{-69}$  M,  $5 \times 10^{-70}$  M,  $10^{-70}$  M,  $5 \times 10^{-71}$  M,  $10^{-71}$  M,  $5 \times 10^{-72}$  M,  $10^{-72}$  M,  $5 \times 10^{-73}$  M,  $10^{-73}$  M,  $5 \times 10^{-74}$  M,  $10^{-74}$  M,  $5 \times 10^{-75}$  M,  $10^{-75}$  M,  $5 \times 10^{-76}$  M,  $10^{-76}$  M,  $5 \times 10^{-77}$  M,  $10^{-77}$  M,  $5 \times 10^{-78}$  M,  $10^{-78}$  M,  $5 \times 10^{-79}$  M,  $10^{-79}$  M,  $5 \times 10^{-80}$  M,  $10^{-80}$  M,  $5 \times 10^{-81}$  M,  $10^{-81}$  M,  $5 \times 10^{-82}$  M,  $10^{-82}$  M,  $5 \times 10^{-83}$  M,  $10^{-83}$  M,  $5 \times 10^{-84}$  M,  $10^{-84}$  M,  $5 \times 10^{-85}$  M,  $10^{-85}$  M,  $5 \times 10^{-86}$  M,  $10^{-86}$  M,  $5 \times 10^{-87}$  M,  $10^{-87}$  M,  $5 \times 10^{-88}$  M,  $10^{-88}$  M,  $5 \times 10^{-89}$  M,  $10^{-89}$  M,  $5 \times 10^{-90}$  M,  $10^{-90}$  M,  $5 \times 10^{-91}$  M,  $10^{-91}$  M,  $5 \times 10^{-92}$  M,  $10^{-92}$  M,  $5 \times 10^{-93}$  M,  $10^{-93}$  M,  $5 \times 10^{-94}$  M,  $10^{-94}$  M,  $5 \times 10^{-95}$  M,  $10^{-95}$  M,  $5 \times 10^{-96}$  M,  $10^{-96}$  M,  $5 \times 10^{-97}$  M,  $10^{-97}$  M,  $5 \times 10^{-98}$  M,  $10^{-98}$  M,  $5 \times 10^{-99}$  M,  $10^{-99}$  M,  $5 \times 10^{-100}$  M,  $10^{-100}$  M,  $5 \times 10^{-101}$  M,  $10^{-101}$  M,  $5 \times 10^{-102}$  M,  $10^{-102}$  M,  $5 \times 10^{-103}$  M,  $10^{-103}$  M,  $5 \times 10^{-104}$  M,  $10^{-104}$  M,  $5 \times 10^{-105}$  M,  $10^{-105}$  M,  $5 \times 10^{-106}$  M,  $10^{-106}$  M,  $5 \times 10^{-107}$  M,  $10^{-107}$  M,  $5 \times 10^{-108}$  M,  $10^{-108}$  M,  $5 \times 10^{-109}$  M,  $10^{-109}$  M,  $5 \times 10^{-110}$  M,  $10^{-110}$  M,  $5 \times 10^{-111}$  M,  $10^{-111}$  M,  $5 \times 10^{-112}$  M,  $10^{-112}$  M,  $5 \times 10^{-113}$  M,  $10^{-113}$  M,  $5 \times 10^{-114}$  M,  $10^{-114}$  M,  $5 \times 10^{-115}$  M,  $10^{-115}$  M,  $5 \times 10^{-116}$  M,  $10^{-116}$  M,  $5 \times 10^{-117}$  M,  $10^{-117}$  M,  $5 \times 10^{-118}$  M,  $10^{-118}$  M,  $5 \times 10^{-119}$  M,  $10^{-119}$  M,  $5 \times 10^{-120}$  M,  $10^{-120}$  M,  $5 \times 10^{-121}$  M,  $10^{-121}$  M,  $5 \times 10^{-122}$  M,  $10^{-122}$  M,  $5 \times 10^{-123}$  M,  $10^{-123}$  M,  $5 \times 10^{-124}$  M,  $10^{-124}$  M,  $5 \times 10^{-125}$  M,  $10^{-125}$  M,  $5 \times 10^{-126}$  M,  $10^{-126}$  M,  $5 \times 10^{-127}$  M,  $10^{-127}$  M,  $5 \times 10^{-128}$  M,  $10^{-128}$  M,  $5 \times 10^{-129}$  M,  $10^{-129}$  M,  $5 \times 10^{-130}$  M,  $10^{-130}$  M,  $5 \times 10^{-131}$  M,  $10^{-131}$  M,  $5 \times 10^{-132}$  M,  $10^{-132}$  M,  $5 \times 10^{-133}$  M,  $10^{-133}$  M,  $5 \times 10^{-134}$  M,  $10^{-134}$  M,  $5 \times 10^{-135}$  M,  $10^{-135}$  M,  $5 \times 10^{-136}$  M,  $10^{-136}$  M,  $5 \times 10^{-137}$  M,  $10^{-137}$  M,  $5 \times 10^{-138}$  M,  $10^{-138}$  M,  $5 \times 10^{-139}$  M,  $10^{-139}$  M,  $5 \times 10^{-140}$  M,  $10^{-140}$  M,  $5 \times 10^{-141}$  M,  $10^{-141}$  M,  $5 \times 10^{-142}$  M,  $10^{-142}$  M,  $5 \times 10^{-143}$  M,  $10^{-143}$  M,  $5 \times 10^{-144}$  M,  $10^{-144}$  M,  $5 \times 10^{-145}$  M,  $10^{-145}$  M,  $5 \times 10^{-146}$  M,  $10^{-146}$  M,  $5 \times 10^{-147}$  M,  $10^{-147}$  M,  $5 \times 10^{-148}$  M,  $10^{-148}$  M,  $5 \times 10^{-149}$  M,  $10^{-149}$  M,  $5 \times 10^{-150}$  M,  $10^{-150}$  M,  $5 \times 10^{-151}$  M,  $10^{-151}$  M,  $5 \times 10^{-152}$  M,  $10^{-152}$  M,  $5 \times 10^{-153}$  M,  $10^{-153}$  M,  $5 \times 10^{-154}$  M,  $10^{-154}$  M,  $5 \times 10^{-155}$  M,  $10^{-155}$  M,  $5 \times 10^{-156}$  M,  $10^{-156}$  M,  $5 \times 10^{-157}$  M,  $10^{-157}$  M,  $5 \times 10^{-158}$  M,  $10^{-158}$  M,  $5 \times 10^{-159}$  M,  $10^{-159}$  M,  $5 \times 10^{-160}$  M,  $10^{-160}$  M,  $5 \times 10^{-161}$  M,  $10^{-161}$  M,  $5 \times 10^{-162}$  M,  $10^{-162}$  M,  $5 \times 10^{-163}$  M,  $10^{-163}$  M,  $5 \times 10^{-164}$  M,  $10^{-164}$  M,  $5 \times 10^{-165}$  M,  $10^{-165}$  M,  $5 \times 10^{-166}$  M,  $10^{-166}$  M,  $5 \times 10^{-167}$  M,  $10^{-167}$  M,  $5 \times 10^{-168}$  M,  $10^{-168}$  M,  $5 \times 10^{-169}$  M,  $10^{-169}$  M,  $5 \times 10^{-170}$  M,  $10^{-170}$  M,  $5 \times 10^{-171}$  M,  $10^{-171}$  M,  $5 \times 10^{-172}$  M,  $10^{-172}$  M,  $5 \times 10^{-173}$  M,  $10^{-173}$  M,  $5 \times 10^{-174}$  M,  $10^{-174}$  M,  $5 \times 10^{-175}$  M,  $10^{-175}$  M,  $5 \times 10^{-176}$  M,  $10^{-176}$  M,  $5 \times 10^{-177}$  M,  $10^{-177}$  M,  $5 \times 10^{-178}$  M,  $10^{-178}$  M,  $5 \times 10^{-179}$  M,  $10^{-179}$  M,  $5 \times 10^{-180}$  M,  $10^{-180}$  M,  $5 \times 10^{-181}$  M,  $10^{-181}$  M,  $5 \times 10^{-182}$  M,  $10^{-182}$  M,  $5 \times 10^{-183}$  M,  $10^{-183}$  M,  $5 \times 10^{-184}$  M,  $10^{-184}$  M,  $5 \times 10^{-185}$  M,  $10^{-185}$  M,  $5 \times 10^{-186}$  M,  $10^{-186}$  M,  $5 \times 10^{-187}$  M,  $10^{-187}$  M,  $5 \times 10^{-188}$  M,  $10^{-188}$  M,  $5 \times 10^{-189}$  M,  $10^{-189}$  M,  $5 \times 10^{-190}$  M,  $10^{-190}$  M,  $5 \times 10^{-191}$  M,  $10^{-191}$  M,  $5 \times 10^{-192}$  M,  $10^{-192}$  M,  $5 \times 10^{-193}$  M,  $10^{-193}$  M,  $5 \times 10^{-194}$  M,  $10^{-194}$  M,  $5 \times 10^{-195}$  M,  $10^{-195}$  M,  $5 \times 10^{-196}$  M,  $10^{-196}$  M,  $5 \times 10^{-197}$  M,  $10^{-197}$  M,  $5 \times 10^{-198}$  M,  $10^{-198}$  M,  $5 \times 10^{-199}$  M,  $10^{-199}$  M,  $5 \times 10^{-200}$  M,  $10^{-200}$  M,  $5 \times 10^{-201}$  M,  $10^{-201}$  M,  $5 \times 10^{-202}$  M,  $10^{-202}$  M,  $5 \times 10^{-203}$  M,  $10^{-203}$  M,  $5 \times 10^{-204}$  M,  $10^{-204}$  M,  $5 \times 10^{-205}$  M,  $10^{-205}$  M,  $5 \times 10^{-206}$  M,  $10^{-206}$  M,  $5 \times 10^{-207}$  M,  $10^{-207}$  M,  $5 \times 10^{-208}$  M,  $10^{-208}$  M,  $5 \times 10^{-209}$  M,  $10^{-209}$  M,  $5 \times 10^{-210}$  M,  $10^{-210}$  M,  $5 \times 10^{-211}$  M,  $10^{-211}$  M,  $5 \times 10^{-212}$  M,  $10^{-212}$  M,  $5 \times 10^{-213}$  M,  $10^{-213}$  M,  $5 \times 10^{-214}$  M,  $10^{-214}$  M,  $5 \times 10^{-215}$  M,  $10^{-215}$  M,  $5 \times 10^{-216}$  M,  $10^{-216}$  M,  $5 \times 10^{-217}$  M,  $10^{-217}$  M,  $5 \times 10^{-218}$  M,  $10^{-218}$  M,  $5 \times 10^{-219}$  M,  $10^{-219}$  M,  $5 \times 10^{-220}$  M,  $10^{-220}$  M,  $5 \times 10^{-221}$  M,  $10^{-221}$  M,  $5 \times 10^{-222}$  M,  $10^{-222}$  M,  $5 \times 10^{-223}$  M,  $10^{-223}$  M,  $5 \times 10^{-224}$  M,  $10^{-224}$  M,  $5 \times 10^{-225}$  M,  $10^{-225}$  M,  $5 \times 10^{-226}$  M,  $10^{-226}$  M,  $5 \times 10^{-227}$  M,  $10^{-227}$  M,  $5 \times 10^{-228}$  M,  $10^{-228}$  M,  $5 \times 10^{-229}$  M,  $10^{-229}$  M,  $5 \times 10^{-230}$  M,  $10^{-230}$  M,  $5 \times 10^{-231}$  M,  $10^{-231}$  M,  $5 \times 10^{-232}$  M,  $10^{-232}$  M,  $5 \times 10^{-233}$  M,  $10^{-233}$  M,  $5 \times 10^{-234}$  M,  $10^{-234}$  M,  $5 \times 10^{-235}$  M,  $10^{-235}$  M,  $5 \times 10^{-236}$  M,  $10^{-236}$  M,  $5 \times 10^{-237}$  M,  $10^{-237}$  M,  $5 \times 10^{-238}$  M,  $10^{-238}$  M,  $5 \times 10^{-239}$  M,  $10^{-239}$  M,  $5 \times 10^{-240}$  M,  $10^{-240}$  M,  $5 \times 10^{-241}$  M,  $10^{-241}$  M,  $5 \times 10^{-242}$  M,  $10^{-242}$  M,  $5 \times 10^{-243}$  M,  $10^{-243}$  M,  $5 \times 10^{-244}$  M,  $10^{-244}$  M,  $5 \times 10^{-245}$  M,  $10^{-245}$  M,  $5 \times 10^{-246}$  M,  $10^{-246}$  M,  $5 \times 10^{-247}$  M,  $10^{-247}$  M,  $5 \times 10^{-248}$  M,  $10^{-248}$  M,  $5 \times 10^{-249}$  M,  $10^{-249}$  M,  $5 \times 10^{-250}$  M,  $10^{-250}$  M,  $5 \times 10^{-251}$  M,  $10^{-251}$  M,  $5 \times 10^{-252}$  M,  $10^{-252}$  M,  $5 \times 10^{-253}$  M,  $10^{-253}$  M,  $5 \times 10^{-254}$  M,  $10^{-254}$  M,  $5 \times 10^{-255}$  M,  $10^{-255}$  M,  $5 \times 10^{-256}$  M,  $10^{-256}$  M,  $5 \times 10^{-257}$  M,  $10^{-257}$  M,  $5 \times 10^{-258}$  M,  $10^{-258}$  M,  $5 \times 10^{-259}$  M,  $10^{-259}$  M,  $5 \times 10^{-260}$  M,  $10^{-260}$  M,  $5 \times 10^{-261}$  M,  $10^{-261}$  M,  $5 \times 10^{-262}$  M,  $10^{-262}$  M,  $5 \times 10^{-263}$  M,  $10^{-263}$  M,  $5 \times 10^{-264}$  M,  $10^{-264}$  M,  $5 \times 10^{-265}$  M,  $10^{-265}$  M,  $5 \times 10^{-266}$  M,  $10^{-266}$  M,  $5 \times 10^{-267}$  M,  $10^{-267}$  M,  $5 \times 10^{-268}$  M,  $10^{-268}$  M,  $5 \times 10^{-269}$  M,  $10^{-269}$  M,  $5 \times 10^{-270}$  M,  $10^{-270}$  M,  $5 \times 10^{-271}$  M,  $10^{-271}$  M,  $5 \times 10^{-272}$  M,  $10^{-272}$  M,  $5 \times 10^{-273}$  M,  $10^{-273}$  M,  $5 \times 10^{-274}$  M,  $10^{-274}$  M,  $5 \times 10^{-275}$  M,  $10^{-275}$  M,  $5 \times 10^{-276}$  M,  $10^{-276}$  M,  $5 \times 10^{-277}$  M,  $10^{-277}$  M,  $5 \times 10^{-278}$  M,  $10^{-278}$  M,  $5 \times 10^{-279}$  M,  $10^{-279}$  M,  $5 \times 10^{-280}$  M,  $10^{-280}$  M,  $5 \times 10^{-281}$  M,  $10^{-281}$  M,  $5 \times 10^{-282}$  M,  $10^{-282}$  M,  $5 \times 10^{-283}$  M,  $10^{-283}$  M,  $5 \times 10^{-284}$  M,  $10^{-284}$  M,  $5 \times 10^{-285}$  M,  $10^{-285}$  M,  $5 \times 10^{-286}$  M,  $10^{-286}$  M,  $5 \times 10^{-287}$  M,  $10^{-287}$  M,  $5 \times 10^{-288}$  M,  $10^{-288}$  M,  $5 \times 10^{-289}$  M,  $10^{-289}$  M,  $5 \times 10^{-290}$  M,  $10^{-290}$  M,  $5 \times 10^{-291}$  M,  $10^{-291}$  M,  $5 \times 10^{-292}$  M,  $10^{-292}$  M,  $5 \times 10^{-293}$  M,  $10^{-293}$  M,  $5 \times 10^{-294}$  M,  $10^{-294}$  M,  $5 \times 10^{-295}$  M,  $10^{-295}$  M,  $5 \times 10^{-296}$  M,  $10^{-296}$  M,  $5 \times 10^{-297}$  M,  $10^{-297}$  M,  $5 \times 10^{-298}$  M,  $10^{-298}$  M,  $5 \times 10^{-299}$  M,  $10^{-299}$  M,  $5 \times 10^{-300}$  M,  $10^{-300}$  M,  $5 \times 10^{-301}$  M,  $10^{-301}$  M,  $5 \times 10^{-302}$  M,  $10^{-302}$  M,  $5 \times 10^{-303}$  M,  $10^{-303}$  M,  $5 \times 10^{-304}$  M,  $10^{-304}$  M,  $5 \times 10^{-305}$  M,  $10^{-305}$  M,  $5 \times 10^{-306}$  M,  $10^{-306}$  M,  $5 \times 10^{-307}$  M,  $10^{-307}$  M,  $5 \times 10^{-308}$  M,  $10^{-308}$  M,  $5 \times 10^{-309}$  M,  $10^{-309}$  M,  $5 \times 10^{-310}$  M,  $10^{-310}$  M,  $5 \times 10^{-311}$  M,  $10^{-311}$  M,  $5 \times 10^{-312}$  M,  $10^{-312}$  M,  $5 \times 10^{-313}$  M,  $10^{-313}$  M,  $5 \times 10^{-314}$  M,  $10^{-314}$  M,  $5 \times 10^{-315}$  M,  $10^{-315}$  M,  $5 \times 10^{-316}$  M,  $10^{-316}$  M,  $5 \times 10^{-317}$  M,  $10^{-317}$  M,  $5 \times 10^{-318}$  M,  $10^{-318}$  M,  $5 \times 10^{-319}$  M,  $10^{-319}$  M,  $5 \times 10^{-320}$  M,  $10^{-320}$  M,  $5 \times 10^{-321}$  M,  $10^{-321}$  M,  $5 \times 10^{-322}$  M,  $10^{-322}$  M,  $5 \times 10^{-323}$  M,  $10^{-323}$  M,  $5 \times 10^{-324}$  M,  $10^{-324}$  M,  $5 \times 10^{-325}$  M,  $10^{-325}$  M,  $5 \times 10^{-326}$  M,  $10^{-326}$  M,  $5 \times 10^{-327}$  M,  $10^{-327}$  M,  $5 \times 10^{-328}$  M,  $10^{-328}$  M,  $5 \times 10^{-329}$  M,  $10^{-329}$  M,  $5 \times 10^{-330}$  M,  $10^{-330}$  M,  $5 \times 10^{-331}$  M,  $10^{-331}$  M,  $5 \times 10^{-332}$  M,  $10^{-332}$  M,  $5 \times 10^{-333}$  M,  $10^{-333}$  M,  $5 \times 10^{-334}$  M,  $10^{-334}$  M,  $5 \times 10^{-335}$  M,  $10^{-335}$  M,  $5 \times 10^{-336}$  M,  $10^{-336}$  M,  $5 \times 10^{-337}$  M,  $10^{-337}$  M,  $5 \times 10^{-338}$  M,  $10^{-338}$  M,  $5 \times 10^{-339}$  M,  $10^{-339}$  M,  $5 \times 10^{-340}$  M,  $10^{-340}$  M,  $5 \times 10^{-341}$  M,  $10^{-341}$  M,  $5 \times 10^{-342}$  M,  $10^{-342}$  M,  $5 \times 10^{-343}$  M,  $10^{-343}$  M,  $5 \times 10^{-344}$  M,  $10^{-344}$  M,  $5 \times 10^{-345}$  M,  $10^{-345}$  M,  $5 \times 10^{-346}$  M,  $10^{-346}$  M,  $5 \times 10^{-347}$  M,  $10^{-347}$  M,  $5 \times 10^{-348}$  M,  $10^{-348}$  M,  $5 \times 10^{-349}$  M,  $10^{-349}$  M,  $5 \times 10^{-350}$  M,  $10^{-350}$  M,  $5 \times 10^{-351}$  M,  $10^{-351}$  M,  $5 \times 10^{-352}$  M,  $10^{-352}$  M,  $5 \times 10^{-353}$  M,  $10^{-353}$  M,  $5 \times 10^{-354}$  M,  $10^{-354}$  M,  $5 \times 10^{-355}$  M,  $10^{-355}$  M,  $5 \times 10^{-356}$  M,  $10^{-356}$  M,  $5 \times 10^{-357}$  M,  $10^{-357}$  M,  $5 \times 10^{-358}$  M,  $10^{-358}$  M,  $5 \times 10^{-359}$  M,  $10^{-359}$  M,  $5 \times 10^{-360}$  M,  $10^{-360}$  M,  $5 \times 10^{-361}$  M,  $10^{-361}$  M,  $5 \times 10^{-362}$  M,  $10^{-362}$  M,  $5 \times 10^{-363}$  M,  $10^{-363}$  M,  $5 \times 10^{-364}$  M,  $10^{-364}$  M,  $5 \times 10^{-365}$  M,  $10^{-365}$  M,  $5 \times 10^{-366}$  M,  $10^{-366}$  M,  $5 \times 10^{-367}$  M,  $10^{-367}$  M,  $5 \times 10^{-368}$  M,  $10^{-368}$  M,  $5 \times 10^{-369}$  M,  $10^{-369}$  M,  $5 \times 10^{-370}$  M,  $10^{-370}$  M,  $5 \times 10^{-371}$  M,  $10^{-371}$  M,  $5 \times 10^{-372}$  M,  $10^{-372}$  M,  $5 \times 10^{-373$

$10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, and  $10^{-15}$  M.

### *Gene Therapy*

[0224] In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, detect, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[0225] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[0226] For general reviews of the methods of gene therapy, see Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, *TIBTECH* 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990).

[0227] In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989).

In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

[0228] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

[0229] In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al.; Nature 342:435-438 (1989)).

[0230] In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the



host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

[0231] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

[0232] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Patent No. 5,436,146).

[0233] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[0234] In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out

by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[0235] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[0236] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[0237] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[0238] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication

WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

[0239] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

#### *Demonstration of Therapeutic or Prophylactic Activity*

[0240] The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

#### *Therapeutic/Prophylactic Administration and Composition*

[0241] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[0242] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional

appropriate formulations and routes of administration can be selected from among those described herein below.

[0243] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0244] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[0245] In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

[0246] In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

[0247] Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

[0248] In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., *Proc. Natl. Acad. Sci. USA* 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0249] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical

carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0250] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0251] The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0252] The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0253] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

[0254] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. Diagnosis and Imaging

[0255] Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases and/or disorders associated with the aberrant expression and/or activity of a

polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

[0256] The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[0257] Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0258] One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally)



to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

[0259] It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99m}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

[0260] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[0261] In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[0262] Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention

include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[0263] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

#### *Kits*

[0264] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

[0265] In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically

synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

[0266] In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

[0267] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[0268] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

[0269] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

[0270] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

### **Fusion Proteins**

[0271] Any CRCGCL polypeptide can be used to generate fusion proteins. For example, the CRCGCL polypeptide, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the CRCGCL polypeptide can be used to indirectly detect the second protein by binding to the CRCGCL. Moreover, because secreted proteins target cellular locations based on trafficking signals, the CRCGCL polypeptides can be used as a targeting molecule once fused to other proteins.

[0272] Examples of domains that can be fused to CRCGCL polypeptides include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

[0273] In certain preferred embodiments, CRCGCL proteins of the invention comprise fusion proteins wherein the CRCGCL polypeptides are those described generally above as m-n, m-n<sup>1</sup>, m<sup>1</sup>-n, and/or m<sup>1</sup>-n<sup>1</sup>. In preferred embodiments, the application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences encoding polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein (e.g., deletions described generally above by the formulas m-n, m-n<sup>1</sup>, m<sup>1</sup>-n, and/or m<sup>1</sup>-n<sup>1</sup>). Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0274] Moreover, fusion proteins may also be engineered to improve characteristics of the CRCGCL polypeptide. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the CRCGCL polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the CRCGCL polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the CRCGCL polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

[0275] As one of skill in the art will appreciate, CRCGCL polypeptides of the present invention, including fragments, and specifically epitope-bearing fragments thereof, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), or albumin (including but not limited to recombinant albumin), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., *Nature* 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995).) Polynucleotides comprising or alternatively consisting of nucleic acids which encode these fusion proteins are also encompassed by the invention.

[0276] Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); K. Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).)

[0277] Moreover, the CRCGCL polypeptides can be fused to marker sequences, such as a peptide which facilitates purification of CRCGCL. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the

fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

[0278] Thus, any of these above fusions can be engineered using the CRCGCL polynucleotides or the polypeptides.

#### **Recombinant and Synthetic Production of CRCGCL**

[0279] The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of polypeptides by recombinant and synthetic techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

[0280] CRCGCL polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

[0281] The CRCGCL polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac*, *trp*, *phoA* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

[0282] As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and

*Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

[0283] Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK223-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

[0284] Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., *Basic Methods In Molecular Biology* (1986). It is specifically contemplated that CRCGCL polypeptides may in fact be expressed by a host cell lacking a recombinant vector.

[0285] CRCGCL polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. For example, see Lin et al., "Purification of Recombinant Human Interferon Beta expressed in *E. coli*" *Methods in Enzymology* 119: 183-192 (1986), which is hereby incorporated by reference in its entirety. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

[0286] CRCGCL polypeptides of the present invention include: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant

techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the CRCGCL polypeptides may be glycosylated or may be non-glycosylated. In addition, CRCGCL polypeptides may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

[0287] In one embodiment, the yeast *Pichia pastoris* is used to express CRCGCL protein in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolism pathway is the oxidation of methanol to formaldehyde using O<sub>2</sub>. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O<sub>2</sub>. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., *et al.*, *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., *et al.*, *Yeast* 5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a CRCGCL polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence may be expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

[0288] In one example, the plasmid vector pPIC9K is used to express DNA encoding a CRCGCL polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector is used to



express and secrete a CRCGCL protein of the invention by virtue of the strong *AOX1* promoter linked to the yeast alpha factor prepro peptide signal sequence (i.e., leader) located upstream of a multiple cloning site.

[0289] Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

[0290] In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a CRCGCL polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

[0291] In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., CRCGCL coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with CRCGCL polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous CRCGCL polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous CRCGCL polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

[0292] In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller, M., et al., 1984, Nature 310:105-111). For example, a peptide corresponding to a fragment of the CRCGCL polypeptides of

the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the CRCGCL polynucleotide sequence. Non-classical amino acids include, but are not limited to, the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0293] The invention encompasses CRCGCL polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease,  $\text{NaBH}_4$ ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

[0294] Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

[0295] Also provided by the invention are chemically modified derivatives of CRCGCL which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U. S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be

modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0296] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

[0297] As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

[0298] The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as

a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

[0299] As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

[0300] One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[0301] As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[0302] One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ( $\text{ClSO}_2\text{CH}_2\text{CF}_3$ ). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

[0303] Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

[0304] The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

[0305] The CRCGCL polypeptides of the invention may be in monomers or multimers (*i.e.*, dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the CRCGCL polypeptides of the invention, their preparation, and compositions (preferably, pharmaceutical compositions) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers,

trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

[0306] Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only CRCGCL polypeptides of the invention (including CRCGCL fragments, variants, splice variants, and fusion proteins, as described herein). These homomers may contain CRCGCL polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only CRCGCL polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing CRCGCL polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing CRCGCL polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing CRCGCL polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

[0307] As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to the CRCGCL polypeptides of the invention. In a specific embodiment, a multimer of the invention contains a type I cytokine receptor or fragments thereof, in addition to CRCGCL polypeptides of the invention. In a further specific embodiment, a multimer of the invention contains the IL-7 alpha receptor chain or fragments thereof in addition to CRCGCL polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

[0308] Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the

heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the CRCGCL polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence ( e.g., that recited in SEQ ID NO:2, or contained in the polypeptide encoded by the clone HTAEK53). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a CRCGCL fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a CRCGCL-Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another Cytokine Receptor family member that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication No. WO 98/49305, the contents of which are herein incorporated by reference in its entirety).

[0309] In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

[0310] Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric

proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

[0311] Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

[0312] In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

[0313] The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide



components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

[0314] Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

[0315] In specific embodiments, CRCGCL polypeptides of the invention are in a heterodimer with a second cytokine receptor chain. In a preferred embodiment, CRCGCL polypeptides of the invention are in a heterodimer with IL-7 receptor alpha chain.

#### **Uses of the CRCGCL Polynucleotides**

[0316] The CRCGCL polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

[0317] There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Using a panel of radiation hybrids, CRCGCL maps to the pseudoautosomal region (PAR) of the sex chromosomes, which is located on both X (Xp22.3) and Y (Yp13.3). Interestingly, two other cytokine receptors map to this region (IL3Ra, and GMCSFRa). See, Kremer et al. "A Cytokine Receptor Gene Cluster in the X-Y

pseudoautosomal region?" Blood 82(1) 22-28 (1993). Thus, CRCGCL polynucleotides can be used in linkage analysis as a marker for the pseudoautosomal region on the X and Y chromosomes.

[0318] Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:1. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human CRCGCL gene corresponding to the SEQ ID NO:1 will yield an amplified fragment.

[0319] Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the CRCGCL polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries, and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety).

[0320] Precise chromosomal location of the CRCGCL polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

[0321] For chromosome mapping, the CRCGCL polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

[0322] The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g., Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000);

and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

[0323] Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library).) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

[0324] Thus, once coinheritance is established, differences in the CRCGCL polynucleotide and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the CRCGCL polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

[0325] Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using CRCGCL polynucleotides. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

[0326] In addition to the foregoing, a CRCGCL polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Both methods rely on binding of the polynucleotide to DNA or RNA. For these techniques, preferred polynucleotides are usually 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).)

Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat and/or prevent disease.

[0327] CRCGCL polynucleotides are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. CRCGCL offers a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

[0328] The CRCGCL polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The CRCGCL polynucleotides can be used as additional DNA markers for RFLP.

[0329] The CRCGCL polynucleotides can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

[0330] Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes,

yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, CRCGCL polynucleotides can be used as polymorphic markers for forensic purposes.

[0331] There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from CRCGCL sequences. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

[0332] Because CRCGCL is found expressed in a cervical cancer cell line (HeLa), activated T cells, and a lung carcinoma cell line (A549), while a shorter variant is also expressed in the lymph node and to a lesser extent in the spleen, CRCGCL polynucleotides are useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to CRCGCL polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). In addition, for a number of disorders of the above tissues or cells, particularly of the immune system, significantly higher or lower levels of CRCGCL gene expression may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" CRCGCL gene expression level, i.e., the CRCGCL expression level in healthy tissue from an individual not having the immune system disorder.

[0333] Thus, the invention provides a diagnostic method useful during diagnosis of a disorder, which involves: (a) assaying CRCGCL gene expression level in cells or body fluid of an individual; (b) comparing the CRCGCL gene expression level with a standard CRCGCL gene expression level, whereby an increase or decrease in the assayed CRCGCL gene expression level compared to the standard expression level is indicative of disorder in the immune system.

[0334] By "assaying CRCGCL gene expression level" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the invention or the level of the mRNA encoding the polypeptide of the invention in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or

relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the related disorder or being determined by averaging levels from a population of individuals not having a related disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

[0335] By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains polypeptide of the present invention or the corresponding mRNA. As indicated, biological samples include body fluids (such as lymph, sera, plasma, urine, semen, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[0336] The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides of the invention are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the invention attached may be used to identify polymorphisms between the isolated polynucleotide sequences of the invention, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, such as for example, in immune system disorders, muscular disorders, neural disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. Such a method is described in U.S. Patents 5,858,659 and 5,856,104. The U.S. Patents referenced *supra* are hereby incorporated by reference in their entirety herein.

[0337] In preferred embodiments, a gene chip with polynucleotides of the invention attached may be used to identify polymorphisms between the isolated polynucleotide sequences of the invention, with polynucleotides isolated from a test subject, and used to indicate detect, treat, prevent, and/or diagnose disorders of the immune system, particularly

relating to the aberrant promotion of B cell lymphopoiesis, or aberrant costimulation of thymic cells or mature T cells.

[0338] The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides of the invention are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, *Science* 254, 1497 (1991); and M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, *Nature* 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point ( $T_{sub.m}$ ) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

[0339] In the very least, the CRCGCL polynucleotides can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

#### Uses of CRCGCL Polypeptides

[0340] CRCGCL polypeptides can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

[0341] CRCGCL polypeptides can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0342] In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

[0343] A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)



[0344] Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of CRCGCL polypeptide in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed CRCGCL polypeptide gene expression level compared to the standard expression level is indicative of a disorder.

[0345] Moreover, CRCGCL polypeptides can be used to treat, detect, and/or prevent disease. For example, patients can be administered CRCGCL polypeptides in an effort to replace absent or decreased levels of the CRCGCL polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B), to inhibit the activity of a polypeptide (e.g., an oncogene), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth).

[0346] Similarly, antibodies directed to CRCGCL polypeptides can also be used to treat, diagnose, detect, and/or prevent disease. For example, administration of an antibody directed to a CRCGCL polypeptide can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

[0347] At the very least, the CRCGCL polypeptides can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. CRCGCL polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, CRCGCL polypeptides can be used to test the following biological activities.

### Gene Therapy Methods

[0348] Another aspect of the present invention is to gene therapy methods for treating disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of the CRCGCL polypeptide of the present invention. This method requires a polynucleotide that codes for a CRCGCL polypeptide operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

[0349] Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a CRCGCL polynucleotide ex vivo, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Beldegrun, A., et al., *J. Natl. Cancer Inst.* 85: 207-216 (1993); Ferrantini, M. et al., *Cancer Research* 53: 1107-1112 (1993); Ferrantini, M. et al., *J. Immunology* 153: 4604-4615 (1994); Kaido, T., et al., *Int. J. Cancer* 60: 221-229 (1995); Ogura, H., et al., *Cancer Research* 50: 5102-5106 (1990); Santodonato, L., et al., *Human Gene Therapy* 7:1-10 (1996); Santodonato, L., et al., *Gene Therapy* 4:1246-1255 (1997); and Zhang, J.-F. et al., *Cancer Gene Therapy* 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells that are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

[0350] As discussed in more detail below, the CRCGCL polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The CRCGCL polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

[0351] In one embodiment, the CRCGCL polynucleotide is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the CRCGCL polynucleotides can also be delivered in

liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

[0352] The CRCGCL polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

[0353] Any strong promoter known to those skilled in the art can be used for driving the expression of CRCGCL DNA. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for CRCGCL.

[0354] Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

[0355] The CRCGCL polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the

interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

[0356] For the naked acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

[0357] The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked CRCGCL DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

[0358] The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

[0359] As is evidenced in the Examples, naked CRCGCL nucleic acid sequences can be administered in vivo results in the successful expression of CRCGCL polypeptide in the femoral arteries of rabbits.

[0360] The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

[0361] In certain embodiments, the CRCGCL polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations.

However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

[0362] Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

[0363] Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

[0364] Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

[0365] For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The

sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

[0366] The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., *Methods of Immunology* (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include  $\text{Ca}^{2+}$ -EDTA chelation (Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483; Wilson et al., *Cell* (1979) 17:77); ether injection (Deamer, D. and Bangham, A., *Biochim. Biophys. Acta* (1976) 443:629; Ostro et al., *Biochem. Biophys. Res. Commun.* (1977) 76:836; Fraley et al., *Proc. Natl. Acad. Sci. USA* (1979) 76:3348); detergent dialysis (Enoch, H. and Strittmatter, P., *Proc. Natl. Acad. Sci. USA* (1979) 76:145); and reverse-phase evaporation (REV) (Fraley et al., *J. Biol. Chem.* (1980) 255:10431; Szoka, F. and Papahadjopoulos, D., *Proc. Natl. Acad. Sci. USA* (1978) 75:145; Schaefer-Ridder et al., *Science* (1982) 215:166), which are herein incorporated by reference.

[0367] Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

[0368] U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

[0369] In certain embodiments, cells are be engineered, ex vivo or in vivo, using a retroviral particle containing RNA which comprises a sequence encoding CRGCL. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

[0370] The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO<sub>4</sub> precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

[0371] The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding CRGCL. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express CRGCL.

[0372] In certain other embodiments, cells are engineered, ex vivo or in vivo, with CRGCL polynucleotide contained in an adenovirus vector. Adenovirus can be manipulated

such that it encodes and expresses CRGCL, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz, A. R. et al. (1974) *Am. Rev. Respir. Dis.* 109:233-238). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991) *Science* 252:431-434; Rosenfeld et al., (1992) *Cell* 68:143-155). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) *Proc. Natl. Acad. Sci. USA* 76:6606).

[0373] Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, *Curr. Opin. Genet. Devel.* 3:499-503 (1993); Rosenfeld et al., *Cell* 68:143-155 (1992); Engelhardt et al., *Human Genet. Ther.* 4:759-769 (1993); Yang et al., *Nature Genet.* 7:362-369 (1994); Wilson et al., *Nature* 365:691-692 (1993); and U.S. Patent No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express E1a and E1b, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

[0374] Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, for example, the HARP promoter of the present invention, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

[0375] In certain other embodiments, the cells are engineered, ex vivo or in vivo, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., *Curr. Topics in Microbiol. Immunol.* 158:97 (1992)). It is also one of the few viruses that may integrate its



DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

[0376] For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The CRCGCL polynucleotide construct is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the CRCGCL polynucleotide construct. These viral particles are then used to transduce eukaryotic cells, either ex vivo or in vivo. The transduced cells will contain the CRCGCL polynucleotide construct integrated into its genome, and will express CRCGCL.

[0377] Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding CRCGCL) via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); and Zijlstra et al., *Nature* 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

[0378] Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the

5' end of the CRCGCL desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

[0379] The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

[0380] The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

[0381] The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous CRCGCL sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous CRCGCL sequence.

[0382] The polynucleotides encoding CRCGCL may be administered along with other polynucleotides encoding angiogenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

[0383] Preferably, the polynucleotide encoding CRCGCL contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

[0384] Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppository solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers (Kaneda et al., Science 243:375 (1989)).

[0385] A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

[0386] Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

[0387] Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

[0388] Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical

delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

[0389] Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian.

[0390] Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

#### **Biological Activities of CRCGCL**

[0391] CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, can be used in assays to test for one or more biological activities. If CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, do exhibit activity in a particular assay, it is likely that CRCGCL may be involved in the diseases associated with the biological activity. Therefore, CRCGCL could be used to treat, diagnose, detect and/or prevent the associated disease.

[0392] CRCGCL is a cell surface receptor homologous to members of the type I cytokine receptor family, and thus should have activity similar to other type I cytokine receptor family members. Current studies in the literature demonstrate that type I cytokine receptors are complexes of several protein chains, and that these heterocomplexes activate the JAK family of cytoplasmic tyrosine kinases as well as STAT proteins -- STAT3, STAT5A and STAT5B, which leads to signalling and activation of B- and/or T-cells. Therefore, CRCGCL may interact specifically with other type I cytokine receptor protein chains (e.g., IL-7 receptor alpha chain), or other B- and T-cell receptor molecules on the surface of B- and T-cells to affect the activation, proliferation, chemotaxis, survival, and/or differentiation of

immune cells. CRCGCL may activate members of the JAK family as well as STAT proteins. CRCGCL may activate STAT5B.

[0393] In a preferred embodiment, CRCGCL specifically interacts with IL-7 receptor alpha chain.

[0394] In a further preferred embodiment, a heterocomplex of CRCGCL and IL-7 receptor alpha chain activates members of the Jax/STAT pathway, including, but not limited to STAT5.

[0395] In a further preferred embodiment, a heterocomplex of CRCGCL and IL-7 receptor alpha chain binds TSLP.

[0396] Antagonists, including but not limited to anti-CRCGCL specific antibodies and/or soluble CRCGCL, may bind to the natural ligand of CRCGCL (e.g., TSLP), bind CRCGCL, and/or bind CRCGCL in complex with other type I cytokine receptors (e.g., IL-7R alpha chain) and antagonize the activity of CRCGCL.

[0397] In a preferred embodiment, soluble CRCGCL (including, but not limited to, the soluble extracellular domain of CRCGCL) binds the natural ligand of CRCGCL (e.g., TSLP) and inhibits the biological activities of CRCGCL (e.g., ability to promote B cell lymphopoiesis; ability to costimulate thymocytes or mature T cells, ability to promote the proliferation, differentiation, and/or chemotaxis of T cells; ability to promote the release of T-cell attracting chemokines from monocytes; ability to enhance the T cell stimulatory capacity of CD11c+ subset of dendritic cells; and/or ability to activate STAT5B).

[0398] In a further preferred embodiment, soluble CRCGCL inhibits B-cell lymphopoiesis, inhibits the costimulation of thymocytes and mature T cells; inhibits the proliferation, differentiation and/or chemotaxis of T cells, inhibits the release of T-cell attracting chemokines from monocytes, inhibits the enhancement of the T cell stimulatory capacity of the CD11c+ subset of dendritic cells, and/or inhibits activation of STAT5B.

[0399] Accordingly, a further preferred embodiment of the invention encompasses the use of CRCGCL antagonists (including, but not limited to, anti-CRCGCL specific antibodies and/or soluble CRCGCL) in the treatment, prevention, detection and/or amelioration of T-cell related disorders, including autoimmune disorders, such as leukemia.

[0400] Binding of CRCGCL induces B-cell lymphopoiesis, and costimulates thymocytes and mature T cells. The binding may occur through homotypic association with membrane bound CRCGCL, homotypic association with membrane bound CRCGCL

complexed with other type I cytokine receptor protein chains (e.g., IL-7 receptor alpha chain), or association with other T- or B-cell receptors. Ligands, such as the natural ligand (e.g., TSLP) and/or antibodies, may mimic the induction of B-cell lymphopoiesis, and costimulate thymocytes and mature T cells by binding to CRCGCL, or other type I cytokine receptors.

[0401] Thus, CRCGCL may be useful as a therapeutic molecule. It could be used to control the proliferation, activation, maturation, survival, and/or differentiation of hematopoietic cells, in particular B- and T-cells. Particularly, CRCGCL may be a useful therapeutic to mediate immune modulation. This control of immune cells would be particularly important in the treatment, diagnosis, detection, and/or prevention of immune disorders, such as autoimmune diseases or immunosuppression, including, but not limited to, immune disorders or diseases as in the section entitled "Immune Activity" below. Preferably, treatment, diagnosis, detection, and/or prevention of immune disorders could be carried out using a secreted form of CRCGCL, gene therapy, or ex vivo applications. Moreover, other inhibitors of CRCGCL, either blocking antibodies or mutant forms, could modulate the expression of CRCGCL. These inhibitors may be useful to treat, diagnose, detect, and/or prevent diseases associated with the misregulation of CRCGCL.

[0402] In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g., CRCGCL polypeptides or anti-CRCGCL antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

[0403] In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., CRCGCL polypeptides or anti-CRCGCL antibodies) in association with toxins or cytotoxic prodrugs.

[0404] By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, cytotoxins (cytotoxic agents), or any molecules or enzymes not normally present in or on the

surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ , or other radioisotopes such as, for example,  $^{103}\text{Pd}$ ,  $^{133}\text{Xe}$ ,  $^{131}\text{I}$ ,  $^{68}\text{Ge}$ ,  $^{57}\text{Co}$ ,  $^{65}\text{Zn}$ ,  $^{85}\text{Sr}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{90}\text{Y}$ ,  $^{153}\text{Sm}$ ,  $^{153}\text{Gd}$ ,  $^{169}\text{Yb}$ ,  $^{51}\text{Cr}$ ,  $^{54}\text{Mn}$ ,  $^{75}\text{Se}$ ,  $^{113}\text{Sn}$ ,  $^{90}\text{Yttrium}$ ,  $^{117}\text{Tin}$ ,  $^{186}\text{Rhenium}$ ,  $^{166}\text{Holmium}$ , and  $^{188}\text{Rhenium}$ ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0405] Techniques known in the art may be applied to label antibodies of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety). A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0406] By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that

may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxylacetamide derivatives of doxorubicin.

[0407] It will be appreciated that conditions caused by a decrease in the standard or normal level of CRCGCL activity in an individual, particularly disorders of the immune system, can be treated by administration of CRCGCL polypeptide (e.g., in the form of soluble extracellular domain or cells expressing the complete protein) or agonist. Thus, the invention also provides a method of treatment of an individual in need of an increased level of CRCGCL activity comprising administering to such an individual a pharmaceutical composition comprising an amount of an isolated CRCGCL polypeptide of the invention, or agonist thereof (e.g., an agonistic CRCGCL antibody), effective to increase the CRCGCL activity level in such an individual.

[0408] It will also be appreciated that conditions caused by a increase in the standard or normal level of CRCGCL activity in an individual, particularly disorders of the immune system, can be treated by administration of CRCGCL polypeptides (e.g., in the form of soluble extracellular domain or cells expressing the complete protein) or antagonist (e.g., an antagonistic CRCGCL antibody). Thus, the invention also provides a method of treatment of an individual in need of an decreased level of CRCGCL activity comprising administering to such an individual a pharmaceutical composition comprising an amount of an isolated CRCGCL polypeptide of the invention, or antagonist thereof, effective to decrease the CRCGCL activity level in such an individual.

[0409] Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated by CRCGCL polynucleotides or polypeptides, agonists and/or antagonists of CRCGCL. Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papilloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-



II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, can be used to treat, prevent, diagnose, and/or detect any of these symptoms or diseases. In specific embodiments, CRCGCL polynucleotides, polypeptides, agonists and/or antagonists are used to treat, prevent, and/or diagnose: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment CRCGCL polynucleotides, polypeptides, agonists and/or antagonists are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment, CRCGCL polynucleotides, polypeptides, or agonists are used to treat, prevent, and/or diagnose AIDS. In an additional specific embodiment CRCGCL polynucleotides, polypeptides, agonists, and/or antagonists are used to treat, prevent, and/or diagnose patients with cryptosporidiosis.

[0410] Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated by CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., *Corynebacterium*, *Mycobacterium*, *Nocardia*), *Cryptococcus neoformans*, Aspergillosis, Bacillaceae (e.g., Anthrax, *Clostridium*), Bacteroidaceae, Blastomycosis, *Bordetella*, *Borrelia* (e.g., *Borrelia burgdorferi*, Brucellosis, Candidiasis, *Campylobacter*, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, *E. coli* (e.g., Enterotoxigenic *E. coli* and Enterohemorrhagic *E. coli*), Enterobacteriaceae (*Klebsiella*, *Salmonella* (e.g., *Salmonella typhi*, and *Salmonella paratyphi*), *Serratia*, *Yersinia*), *Erysipelothrix*, *Helicobacter*, Legionellosis, Leptospirosis, *Listeria* (e.g., *Listeria monocytogenes*), Mycoplasmatales, *Mycobacterium leprae*, *Vibrio cholerae*, Neisseriaceae (e.g., *Acinetobacter*, Gonorrhea, Meningococcal), *Meisseria meningitidis*, Pasteurellaceae Infections (e.g., *Actinobacillus*, *Haemophilus* (e.g., *Haemophilus influenza* type B), *Pasteurella*), *Pseudomonas*, Rickettsiaceae, Chlamydiaceae, Syphilis,

Shigella spp., Staphylococcal, Meningococcal, Pneumococcal and Streptococcal (e.g., Streptococcus pneumoniae and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., meningitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. CRCGCL polynucleotides, polypeptides, agonists and/or antagonists of CRCGCL, can be used to treat, prevent, diagnose, and/or detect any of these symptoms or diseases. In specific embodiments, CRCGCL polynucleotides, polypeptides, agonists and/or antagonists thereof are used to treat, prevent, and/or diagnose: tetanus, Diphtheria, botulism, and/or meningitis type B.

[0411] Moreover, parasitic agents causing disease or symptoms that can be treated by CRCGCL polynucleotides, polypeptides, agonists and/or antagonists of CRCGCL, include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. CRCGCL polynucleotides, polypeptides, agonists and/or antagonists of CRCGCL, can be used to treat, prevent, diagnose, and/or detect any of these symptoms or diseases. In specific embodiments, CRCGCL polynucleotides, polypeptides, agonists and/or antagonists thereof are used to treat, prevent, and/or diagnose malaria.

[0412] In another embodiment, CRCGCL polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, are used to treat, prevent, and/or

diagnose inner ear infection (such as, for example, otitis media), as well as other infections characterized by infection with *Streptococcus pneumoniae* and other pathogenic organisms.

[0413] In a specific embodiment, CRCGCL polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-CRCGCL antibodies) are used to treat, detect, diagnose and/or prevent a disorder characterized by deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, CRCGCL polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-CRCGCL antibodies) may be used to treat, detect, diagnose and/or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carinii.

[0414] CRCGCL polynucleotides or polypeptides of the invention, or agonists or antagonists thereof, may be used to diagnose, prognose, treat or prevent one or more of the following diseases or disorders, or conditions associated therewith: primary immunodeficiencies, immune-mediated thrombocytopenia, Kawasaki syndrome, bone marrow transplant (e.g., recent bone marrow transplant in adults or children), chronic B-cell lymphocytic leukemia, HIV infection (e.g., adult or pediatric HIV infection), chronic inflammatory demyelinating polyneuropathy, and post-transfusion purpura.

[0415] Additionally, CRCGCL polynucleotides or polypeptides of the invention, or agonists or antagonists thereof, may be used to diagnose, prognose, treat or prevent one or more of the following diseases, disorders, or conditions associated therewith, Guillain-Barre syndrome, anemia (e.g., anemia associated with parvovirus B19, patients with stable multiple myeloma who are at high risk for infection (e.g., recurrent infection), autoimmune hemolytic anemia (e.g., warm-type autoimmune hemolytic anemia), thrombocytopenia (e.g., neonatal thrombocytopenia), and immune-mediated neutropenia), transplantation (e.g., cytomegalovirus (CMV)-negative recipients of CMV-positive organs), hypogammaglobulinemia (e.g., hypogammaglobulinemic neonates with risk factor for infection or morbidity), epilepsy (e.g., intractable epilepsy), systemic vasculitic syndromes,

myasthenia gravis (e.g. decompensation in myasthenia gravis), dermatomyositis, and polymyositis.

[0416] Additional preferred embodiments of the invention include, but are not limited to, the use of CRCGCL polypeptides, CRCGCL polynucleotides, and functional agonists and/or antagonists thereof, in the following applications:

[0417] Administration to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to inhibit the immune system to produce decreased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to inhibit higher affinity antibody production (e.g., IgG, IgA, IgM, and IgE), and/or to decrease an immune response. In a specific nonexclusive embodiment, CRCGCL polypeptides of the invention, and/or antagonists thereof, are administered to inhibit the immune system to produce decreased quantities of IgG. In another specific nonexclusive embodiment, CRCGCL polypeptides of the invention and/or agonists thereof, are administered to boost the immune system to produce decreased quantities of IgA. In another specific nonexclusive embodiment, CRCGCL polypeptides of the invention and/or antagonists thereof, are administered to inhibit the immune system to produce decreased quantities of IgM.

[0418] As an agent that reduces the immune status of an individual prior to their receipt of immunosuppressive therapies.

[0419] As an agent to decrease serum immunoglobulin concentrations.

[0420] As an immune system inhibitor prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation.

[0421] As an agent to reduce immunoresponsiveness. B cell immunodeficiencies that may be ameliorated or treated by administering CRCGCL agonists of the invention include, but are not limited to, severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified

hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVID) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

[0422] CRGCL agonists may be used as agents to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the CRGCL agonists of the invention include, but are not limited to, HIV Infection, AIDS, bone marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

[0423] CRGCL agonists may be used as agents to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering CRGCL agonists include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, recovery from surgery.

[0424] As an agent to direct an individual's immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

[0425] As a means to inhibit tumor proliferation.

[0426] As a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect.

[0427] As a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence such as observed among SCID patients.

[0428] As an antigen for the generation of antibodies to inhibit or enhance CRCGCL-mediated responses.

[0429] As a means of inhibiting monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leshmania.

[0430] As pretreatment of bone marrow samples prior to transplant. Such treatment would decrease B cell representation and thus modulate recovery.

[0431] CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists may be used to modulate IgE concentrations in vitro or in vivo.

[0432] Additionally, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, may be used to treat, prevent, and/or diagnose IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema.

[0433] In a specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate selective IgA deficiency.

[0434] In another specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate ataxia-telangiectasia.

[0435] In another specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate common variable immunodeficiency.

[0436] In another specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate X-linked agammaglobulinemia.

[0437] In another specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate severe combined immunodeficiency (SCID).

[0438] In another specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate Wiskott-Aldrich syndrome.

[0439] In another specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate X-linked Ig deficiency with hyper IgM.

[0440] In another specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, or antagonists or antagonists (e.g., anti-CRCGCL antibodies) thereof, is administered to treat, prevent, and/or diagnose chronic myelogenous leukemia, acute myelogenous leukemia, leukemia, hystiocytic leukemia, monocytic leukemia (e.g., acute monocytic leukemia), leukemic reticulosis, Shilling Type monocytic leukemia, and/or other leukemias derived from monocytes and/or monocytic cells and/or tissues.

[0441] In another specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate monocytic leukemoid reaction, as seen, for example, with tuberculosis.

[0442] In another specific embodiment, CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate monocytic leukocytosis, monocytic leukopenia, monocytopenia, and/or monocytosis.

[0443] In a specific embodiment, CRCGCL polynucleotides or polypeptides of the invention, and/or anti-CRCGCL antibodies and/or agonists or antagonists thereof, are used to treat, prevent, detect, and/or diagnose primary B lymphocyte disorders and/or diseases, and/or conditions associated therewith.

[0444] In a preferred embodiment, CRCGCL polynucleotides, polypeptides, and/or agonists and/or antagonists thereof are used to treat, prevent, and/or diagnose diseases or disorders affecting or conditions associated with any one or more of the various mucous membranes of the body. Such diseases or disorders include, but are not limited to, for example, mucositis, mucoclasia, mucocolitis, mucocutaneous leishmaniasis (such as, for example, American leishmaniasis, leishmaniasis americana, nasopharyngeal leishmaniasis, and New World leishmaniasis), mucocutaneous lymph node syndrome (for example, Kawasaki disease), mucoenteritis, mucoepidermoid carcinoma, mucoepidermoid tumor, mucoepithelial dysplasia, mucoid adenocarcinoma, mucoid degeneration, myxoid degeneration; myxomatous degeneration; myxomatosis, mucoid medial degeneration (for example, cystic medial necrosis), mucolipidosis (including, for example, mucolipidosis I, mucolipidosis II, mucolipidosis III, and mucolipidosis IV), mucolysis disorders,

mucomembranous enteritis, mucoenteritis, mucopolysaccharidosis (such as, for example, type I mucopolysaccharidosis (i.e., Hurler's syndrome), type IS mucopolysaccharidosis (i.e., Scheie's syndrome or type V mucopolysaccharidosis), type II mucopolysaccharidosis (i.e., Hunter's syndrome), type III mucopolysaccharidosis (i.e., Sanfilippo's syndrome), type IV mucopolysaccharidosis (i.e., Morquio's syndrome), type VI mucopolysaccharidosis (i.e., Maroteaux-Lamy syndrome), type VII mucopolysaccharidosis (i.e., mucopolysaccharidosis due to beta-glucuronidase deficiency), and mucosulfatidosis), mucopolysacchariduria, mucopurulent conjunctivitis, mucopus, mucormycosis (i.e., zygomycosis), mucosal disease (i.e., bovine virus diarrhea), mucous colitis (such as, for example, mucocolitis and myxomembranous colitis), and mucoviscidosis (such as, for example, cystic fibrosis, cystic fibrosis of the pancreas, Clarke-Hadfield syndrome, fibrocystic disease of the pancreas, mucoviscidosis, and viscidosis). In a highly preferred embodiment, CRCGCL polynucleotides, polypeptides, and/or agonists and/or antagonists thereof are used to treat, prevent, and/or diagnose mucositis, especially as associated with chemotherapy.

[0445] In a preferred embodiment, CRCGCL polynucleotides, polypeptides, and/or agonists and/or antagonists thereof are used to treat, prevent, and/or diagnose diseases or disorders affecting or conditions associated with sinusitis.

[0446] An additional condition, disease or symptom that can be treated, prevented, and/or diagnosed by CRCGCL polynucleotides or polypeptides, agonists and/or antagonists of CRCGCL, is osteomyelitis.

[0447] An additional condition, disease or symptom that can be treated, prevented, and/or diagnosed by CRCGCL polynucleotides or polypeptides, agonists and/or antagonists of CRCGCL, is endocarditis.

[0448] All of the above described applications as they may apply to veterinary medicine.

[0449] CRCGCL agonists and/or antagonists may be used as a therapy for B cell malignancies such as ALL, Hodgkins disease, non-Hodgkins lymphoma, Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, and EBV-transformed diseases, as well as a therapy for chronic hypergammaglobulinemia evident in such diseases as monoclonalgammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoclonalgammopathies, and plasmacytomas.

[0450] An immunosuppressive agent(s).



[0451] CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists may be used to modulate IgE concentrations in vitro or in vivo.

[0452] In another embodiment, administration of CRCGCL polypeptides or polynucleotides of the invention, agonists and/or antagonists thereof, may be used to treat, prevent, and/or diagnose IgE-mediated allergic reactions including, but not limited to, asthma, rhinitis, and eczema.

[0453] The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

[0454] The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

[0455] All of the above described applications as they may apply to veterinary medicine. Moreover, all applications described herein may also apply to veterinary medicine.

[0456] CRCGCL polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, may be used to treat, prevent, and/or diagnose various immune system-related disorders and/or conditions associated with these disorders, in mammals, preferably humans. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of CRCGCL polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof that can inhibit an immune response, particularly the proliferation of B cells and/or the production of immunoglobulins, may be an effective therapy in treating and/or preventing autoimmune disorders. Thus, in preferred embodiments, CRCGCL agonists and/or antagonists of the invention are used to treat, prevent, and/or diagnose an autoimmune disorder.

[0457] Autoimmune disorders and conditions associated with these disorders that may be treated, prevented, and/or diagnosed with the CRCGCL polynucleotides, polypeptides, agonists and/or antagonists of the invention, include, but are not limited to, autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, idiopathic

thrombocytopenia purpura, autoimmunocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g. IgA nephropathy), Multiple Sclerosis, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henloch-Scoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease.

[0458] Additional autoimmune disorders (that are highly probable) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis) (often characterized, e.g., by cell-mediated and humoral thyroid cytotoxicity), systemic lupus erythematosus (often characterized, e.g., by circulating and locally generated immune complexes), Goodpasture's syndrome (often characterized, e.g., by anti-basement membrane antibodies), Pemphigus (often characterized, e.g., by epidermal acantholytic antibodies), Receptor autoimmunities such as, for example, (a) Graves' Disease (often characterized, e.g., by TSH receptor antibodies), (b) Myasthenia Gravis (often characterized, e.g., by acetylcholine receptor antibodies), and (c) insulin resistance (often characterized, e.g., by insulin receptor antibodies), autoimmune hemolytic anemia (often characterized, e.g., by phagocytosis of antibody-sensitized RBCs), autoimmune thrombocytopenic purpura (often characterized, e.g., by phagocytosis of antibody-sensitized platelets).

[0459] Additional autoimmune disorders (that are probable) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, rheumatoid arthritis (often characterized, e.g., by immune complexes in joints), scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis/dermatomyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized, e.g., by glomerular basement membrane antibodies or immune complexes) such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid (often characterized, e.g., by

IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

[0460] Additional autoimmune disorders (that are possible) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondrial antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiomyopathy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), inflammatory myopathies, and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

[0461] In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, and/or diagnosed using anti-CRCGCL antibodies.

[0462] In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed using CRCGCL, agonists and/or antagonists of the invention.

[0463] In a specific preferred embodiment, lupus is treated, prevented, and/or diagnosed using CRCGCL, agonists and/or antagonists of the invention.

[0464] In a specific preferred embodiment, nephritis associated with lupus is treated, prevented, and/or diagnosed using CRCGCL, agonists and/or antagonists of the invention.

[0465] Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, and/or diagnosed by CRCGCL polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof. Moreover, these molecules can be used to treat, prevent, and/or

diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

[0466] CRCGCL polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, may also be used to treat, prevent, and/or diagnose organ rejection or graft-versus-host disease (GVHD) and/or conditions associated therewith. Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of CRCGCL polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

[0467] Similarly, CRCGCL polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, may also be used to modulate inflammation. For example, CRCGCL polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat, prevent, and/or diagnose inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

[0468] In a specific embodiment, anti-CRCGCL antibodies of the invention are used to treat, prevent, modulate, detect, and/or diagnose inflammation.

[0469] In a specific embodiment, anti-CRCGCL antibodies of the invention are used to treat, prevent, modulate, detect, and/or diagnose inflammatory disorders.

[0470] In another specific embodiment, anti-CRCGCL antibodies of the invention are used to treat, prevent, modulate, detect, and/or diagnose allergy and/or hypersensitivity.

[0471] In a specific embodiment, CRCGCL polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, are used to treat, prevent, and/or diagnose chronic obstructive pulmonary disease (COPD).

[0472] In another embodiment, CRCGCL polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, are used to treat, prevent, and/or diagnose fibroses and conditions associated with fibroses, such as, for example, but not limited to, cystic fibrosis (including such fibroses as cystic fibrosis of the pancreas, Clarke-Hadfield syndrome, fibrocystic disease of the pancreas, mucoviscidosis, and viscidosis), endomyocardial fibrosis, idiopathic retroperitoneal fibrosis, leptomeningeal fibrosis, mediastinal fibrosis, nodular subepidermal fibrosis, pericentral fibrosis, perimuscular fibrosis, pipestem fibrosis, replacement fibrosis, subadventitial fibrosis, and Symmers' clay pipestem fibrosis.

[0473] Diseases associated with increased cell survival, or the inhibition of apoptosis that may be diagnosed, treated, or prevented with the CRCGCL polynucleotides or polypeptides of the invention, and agonists and antagonists thereof, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to, colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as systemic lupus erythematosus and immune-related glomerulonephritis rheumatoid arthritis); viral infections (such as herpes viruses, pox viruses and adenoviruses); inflammation; graft vs. host disease; acute graft rejection and chronic graft rejection. Thus, in preferred embodiments CRCGCL polynucleotides or polypeptides of the invention and/or agonists or antagonists thereof, are used to treat, prevent, and/or diagnose autoimmune diseases and/or inhibit the growth, progression, and/or metastasis of cancers, including, but not limited to, those cancers disclosed herein, such as, for example, lymphocytic leukemias (including, for example, MLL and chronic lymphocytic leukemia (CLL)) and follicular lymphomas. In another embodiment CRCGCL polynucleotides or polypeptides of the invention are used to activate, differentiate or proliferate cancerous cells or tissue (e.g., B cell lineage related cancers (e.g., CLL and MLL), lymphocytic leukemia, or lymphoma) and thereby render the cells more vulnerable to cancer therapy (e.g., chemotherapy or radiation therapy).

[0474] Moreover, in other embodiments, CRCGCL polynucleotides or polypeptides of the invention or agonists or antagonists thereof, are used to inhibit the growth, progression,

and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

[0475] Diseases associated with increased apoptosis that may be diagnosed, treated, or prevented with the CRCGCL polynucleotides or polypeptides of the invention, and agonists and antagonists thereof, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration); myelodysplastic syndromes (such as aplastic anemia), ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia. Thus, in preferred embodiments CRCGCL polynucleotides or polypeptides of the invention and/or agonists or antagonists thereof, are used to treat, prevent, and/or diagnose the diseases and disorders listed above.

[0476] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in the diagnosis and treatment or prevention of a wide range of diseases and/or conditions. Such diseases and conditions include, but are not limited to,

cancer (e.g., immune cell related cancers, breast cancer, prostate cancer, ovarian cancer, follicular lymphoma, cancer associated with mutation or alteration of p53, brain tumor, bladder cancer, uterocervical cancer, colon cancer, colorectal cancer, non-small cell carcinoma of the lung, small cell carcinoma of the lung, stomach cancer, etc.), lymphoproliferative disorders (e.g., lymphadenopathy), microbial (e.g., viral, bacterial, etc.) infection (e.g., HIV-1 infection, HIV-2 infection, herpesvirus infection (including, but not limited to, HSV-1, HSV-2, CMV, VZV, HHV-6, HHV-7, EBV), adenovirus infection, poxvirus infection, human papilloma virus infection, hepatitis infection (e.g., HAV, HBV, HCV, etc.), *Helicobacter pylori* infection, invasive *Staphylococci*, etc.), parasitic infection, nephritis, bone disease (e.g., osteoporosis), atherosclerosis, pain, cardiovascular disorders (e.g., neovascularization, hypovascularization or reduced circulation (e.g., ischemic disease (e.g., myocardial infarction, stroke, etc.)), AIDS, allergy, inflammation, neurodegenerative disease (e.g., Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, pigmentary retinitis, cerebellar degeneration, etc.), graft rejection (acute and chronic), graft vs. host disease, diseases due to osteomyelodysplasia (e.g., aplastic anemia, etc.), joint tissue destruction in rheumatism, liver disease (e.g., acute and chronic hepatitis, liver injury, and cirrhosis), autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis.

[0477] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in promoting angiogenesis, wound healing (e.g., wounds, burns, and bone fractures). Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are also useful as an adjuvant to enhance immune responsiveness to specific antigen, anti-viral immune responses.

[0478] More generally, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in regulating (i.e., elevating or reducing) immune response. For example, polynucleotides and/or polypeptides of the invention may be useful in preparation or recovery from surgery, trauma, radiation therapy, chemotherapy, and transplantation, or may be used to boost immune response and/or recovery in the elderly and

immunocompromised individuals. Alternatively, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful as immunosuppressive agents, for example in the treatment or prevention of autoimmune disorders. In specific embodiments, polynucleotides and/or polypeptides of the invention are used to treat or prevent chronic inflammatory, allergic or autoimmune conditions, such as those described herein or are otherwise known in the art.

[0479] Preferably, treatment using CRCGCL polynucleotides or polypeptides, and/or agonists or antagonists of CRCGCL (e.g., anti-CRCGCL antibody), could either be by administering an effective amount of CRCGCL polypeptide of the invention, or agonist or antagonist thereof, to the patient, or by removing cells from the patient, supplying the cells with CRCGCL polynucleotide, and returning the engineered cells to the patient (ex vivo therapy). Moreover, as further discussed herein, the CRCGCL polypeptide or polynucleotide can be used as an adjuvant in a vaccine to raise an immune response against infectious disease.

#### **Immune Activity**

[0480] CRCGCL polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of CRCGCL, may be useful in treating, preventing, and/or diagnosing diseases, deficiencies, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, deficiencies, disorders, and/or conditions may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, CRCGCL polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of CRCGCL, can be used as a marker or detector of a particular immune system disease or disorder.

[0481] Interestingly, CRCGCL maps to the pseudoautosomal regions on the X and Y chromosomes. It is likely that mutations in CRCGCL may also lead to immune disorders, especially those involving activated T cells. Moreover, mutations in CRCGCL may be involved in autoimmune diseases, especially X-linked autoimmune diseases.



**[0482]** Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing immunodeficiencies, including both congenital and acquired immunodeficiencies. Examples of B cell immunodeficiencies in which immunoglobulin levels B cell function and/or B cell numbers are decreased include: X-linked agammaglobulinemia (Bruton's disease), X-linked infantile agammaglobulinemia, X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, X-linked lymphoproliferative syndrome (XLP), agammaglobulinemia including congenital and acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, unspecified hypogammaglobulinemia, recessive agammaglobulinemia (Swiss type), Selective IgM deficiency, selective IgA deficiency, selective IgG subclass deficiencies, IgG subclass deficiency (with or without IgA deficiency), Ig deficiency with increased IgM, IgG and IgA deficiency with increased IgM, antibody deficiency with normal or elevated Igs, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), common variable immunodeficiency (CVID), common variable immunodeficiency (CVI) (acquired), and transient hypogammaglobulinemia of infancy.

**[0483]** In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof.

**[0484]** Examples of congenital immunodeficiencies in which T cell and/or B cell function and/or number is decreased include, but are not limited to: DiGeorge anomaly, severe combined immunodeficiencies (SCID) (including, but not limited to, X-linked SCID, autosomal recessive SCID, adenosine deaminase deficiency, purine nucleoside phosphorylase (PNP) deficiency, Class II MHC deficiency (Bare lymphocyte syndrome), Wiskott-Aldrich syndrome, and ataxia telangiectasia), thymic hypoplasia, third and fourth pharyngeal pouch syndrome, 22q11.2 deletion, chronic mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia, immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity.

**[0485]** In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are ameliorated or treated by, for example, administering the polypeptides or polynucleotides of the invention, or antagonists or agonists thereof.

[0486] Other immunodeficiencies that may be ameliorated or treated by administering polypeptides or polynucleotides of the invention, and/or agonists thereof, include, but are not limited to, Chronic granulomatous disease, Chédiak-Higashi syndrome, Myeloperoxidase deficiency, Leukocyte glucose-6-phosphate dehydrogenase Deficiency, X-linked lymphoproliferative syndrome (XLP), leukocyte adhesion deficiency, complement component deficiencies (including C1, C2, C3, C4, C5, C6, C7, C8 and/or C9 deficiencies), reticular dysgenesis, thymic aplasia-aplasia, immunodeficiency with thymoma, severe congenital leukopenia, dysplasia with immunodeficiency, neonatal neutropenia, short limbed dwarfism, and Nezelof syndrome-combined immunodeficiency with Igs.

[0487] In a preferred embodiment, the immunodeficiencies and/or conditions associated with the immunodeficiencies recited above are treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

[0488] In a preferred embodiment polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among immunodeficient individuals. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

[0489] The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides and polypeptides of the invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

[0490] Autoimmune diseases or disorders that may be treated, prevented, and/or diagnosed by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, one or more of the following: systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis, autoimmune thyroiditis, Hashimoto's thyroiditis, autoimmune hemolytic anemia, hemolytic

anemia, thrombocytopenia, autoimmune thrombocytopenia purpura, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, purpura (e.g., Henoch-Schoenlein purpura), autoimmune cytopenia, Goodpasture's syndrome, Pemphigus vulgaris, myasthenia gravis, Grave's disease (hyperthyroidism), and insulin-resistant diabetes mellitus.

[0491] Additional disorders that are likely to have an autoimmune component that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, type II collagen-induced arthritis, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Autism, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye.

[0492] Additional disorders that are likely to have an autoimmune component that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized, e.g., by glomerular basement membrane antibodies or immune complexes), bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

[0493] Additional disorders that may have an autoimmune component that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondrial antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some

cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiomy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

[0494] In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, and/or diagnosed using for example, antagonists or agonists, polypeptides or polynucleotides, or antibodies of the present invention. In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, systemic lupus erythematosus is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, idiopathic thrombocytopenia purpura is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment IgA nephropathy is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

[0495] In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention

[0496] In preferred embodiments, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a immunosuppressive agent(s).

[0497] CRGCL polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or detecting deficiencies or disorders of hematopoietic cells. CRGCL polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention, could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a

decrease in certain (or many) types hematopoietic cells, including but not limited to, leukopenia, neutropenia, anemia, thrombocytopenia. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a increase in certain (or many) types hematopoietic cells, including but not limited to, histiocytosis.

[0498] Allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, and/or diagnosed using polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof. Moreover, these molecules can be used to treat, prevent, and/or diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

[0499] Additionally, polypeptides or polynucleotides of the invention, and/or agonists thereof, may be used to treat or prevent IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate IgE concentrations *in vitro* or *in vivo*.

[0500] Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention have uses in the diagnosis, prognosis, prevention, and/or treatment of inflammatory conditions. For example, since polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists of the invention may inhibit the activation, proliferation and/or differentiation of cells involved in an inflammatory response, these molecules can be used to diagnose, prognose, prevent, and/or treat chronic and acute inflammatory conditions. Such inflammatory conditions include, but are not limited to, for example, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome), ischemia-reperfusion injury, endotoxin lethality, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, over production of cytokines (e.g., TNF or IL-1.), respiratory disorders (such as, e.g., asthma and allergy); gastrointestinal disorders (such as, e.g., inflammatory bowel disease); cancers (such as, e.g., gastric, ovarian, lung, bladder, liver, and breast); CNS disorders (such as, e.g., multiple sclerosis; ischemic brain injury and/or stroke; traumatic brain injury; neurodegenerative disorders, such as, e.g.,

Parkinson's disease and Alzheimer's disease; AIDS-related dementia; and prion disease); cardiovascular disorders (such as, e.g., atherosclerosis, myocarditis, cardiovascular disease, and cardiopulmonary bypass complications); as well as many additional diseases, conditions, and disorders that are characterized by inflammation (such as, e.g., hepatitis, rheumatoid arthritis, gout, trauma, pancreatitis, sarcoidosis, dermatitis, renal ischemia-reperfusion injury, Grave's disease, systemic lupus erythematosus, diabetes mellitus, and allogenic transplant rejection).

[0501] Because inflammation is a fundamental defense mechanism, inflammatory disorders can effect virtually any tissue of the body. Accordingly, polynucleotides, polypeptides, and antibodies of the invention, as well as agonists or antagonists thereof, have uses in the treatment of tissue-specific inflammatory disorders, including, but not limited to, adrenalitis, alveolitis, angiocholecystitis, appendicitis, balanitis, blepharitis, bronchitis, bursitis, carditis, cellulitis, cervicitis, cholecystitis, chorditis, cochitis, colitis, conjunctivitis, cystitis, dermatitis, diverticulitis, encephalitis, endocarditis, esophagitis, eustachitis, fibrositis, folliculitis, gastritis, gastroenteritis, gingivitis, glossitis, hepatosplenitis, keratitis, labyrinthitis, laryngitis, lymphangitis, mastitis, media otitis, meningitis, metritis, mucitis, myocarditis, myositis, myringitis, nephritis, neuritis, orchitis, osteochondritis, otitis, pericarditis, peritendonitis, peritonitis, pharyngitis, phlebitis, poliomyelitis, prostatitis, pulpitis, retinitis, rhinitis, salpingitis, scleritis, sclerochoroiditis, scrotitis, sinusitis, sponylitis, steatitis, stomatitis, synovitis, syringitis, tendonitis, tonsillitis, urethritis, and vaginitis.

[0502] In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to treat, diagnose, and/or prevent organ transplant rejections and graft-versus-host disease. Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. Polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD. In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation,

differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing experimental allergic and hyperacute xenograft rejection.

[0503] In other embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to treat, diagnose, and/or prevent immune complex diseases, including, but not limited to, serum sickness, post streptococcal glomerulonephritis, and polyarteritis nodosa, immune complex-induced vasculitis,

[0504] Polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention can be used to treat, detect, and/or prevent infectious agents. For example, by increasing the immune response, particularly increasing the proliferation activation and/or differentiation of B and/or T cells, infectious diseases may be treated, detected, and/or prevented. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also directly inhibit the infectious agent (refer to section of application listing infectious agents, etc); without necessarily eliciting an immune response.

[0505] In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a vaccine adjuvant that enhances immune responsiveness to specific antigen. In a specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance tumor-specific immune responses.

[0506] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, Respiratory syncytial virus, Dengue, Rotavirus, Japanese B encephalitis, Influenza A and B, Parainfluenza, Measles,

Cytomegalovirus, Rabies, Junin, Chikungunya, Rift Valley fever, Herpes simplex, and yellow fever.

[0507] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B.

[0508] In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Meissneria meningitidis*, *Streptococcus pneumoniae*, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, and *Borrelia burgdorferi*.

[0509] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to Plasmodium (malaria) or Leishmania.

[0510] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed to treat infectious diseases including silicosis, sarcoidosis, idiopathic pulmonary fibrosis by, for example, by preventing the recruitment and activation of mononuclear phagocytes.

[0511] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an antigen for the



generation of antibodies to inhibit or enhance immune mediated responses against polypeptides of the invention.

[0512] In one embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are administered to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production and immunoglobulin class switching (e.g., IgG, IgA, IgM, and IgE), and/or to increase an immune response.

[0513] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a stimulator of B cell responsiveness to pathogens.

[0514] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an activator of T cells.

[0515] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

[0516] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to induce higher affinity antibodies.

[0517] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to increase serum immunoglobulin concentrations.

[0518] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to accelerate recovery of immunocompromised individuals.

[0519] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among aged populations.

[0520] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g.,

allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

[0521] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, HIV Infection, AIDS, bone marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

[0522] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, and recovery from surgery.

[0523] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention enhance antigen presentation or antagonizes antigen presentation in vitro or in vivo. Moreover, in related embodiments, said enhancement or antagonization of antigen presentation may be useful as an anti-tumor treatment or to modulate the immune system.

[0524] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to direct an

individual's immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

[0525] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

[0526] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodeficiency.

[0527] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect. In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as pretreatment of bone marrow samples prior to transplant.

[0528] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence/immunodeficiency such as observed among SCID patients.

[0529] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leshmania.

[0530] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of regulating secreted cytokines that are elicited by polypeptides of the invention.

[0531] All of the above described applications as they may apply to veterinary medicine.

[0532] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of blocking various aspects of immune responses to foreign agents or self. Examples of diseases or conditions in which blocking of certain aspects of immune responses may be desired include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and diseases/disorders associated with pathogens.

[0533] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythramatosus and multiple sclerosis.

[0534] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a inhibitor of B and/or T cell migration in endothelial cells. This activity disrupts tissue architecture or cognate responses and is useful, for example in disrupting immune responses, and blocking sepsis.

[0535] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for chronic hypergammaglobulinemia evident in such diseases as monoclonalgammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoclonalgammopathies, and plasmacytomas.

[0536] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed for instance to inhibit polypeptide chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain autoimmune and chronic inflammatory and infective diseases. Examples of autoimmune diseases are described herein and include multiple sclerosis, and insulin-dependent diabetes.

[0537] The polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed to treat idiopathic hyper-eosinophilic syndrome by, for example, preventing eosinophil production and migration.

[0538] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used to enhance or inhibit complement mediated cell lysis.

[0539] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used to enhance or inhibit antibody dependent cellular cytotoxicity.

[0540] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed for treating atherosclerosis, for example, by preventing monocyte infiltration in the artery wall.

[0541] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed to treat adult respiratory distress syndrome (ARDS).

[0542] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be useful for stimulating wound and tissue repair, stimulating angiogenesis, stimulating the repair of vascular or lymphatic diseases or disorders. Additionally, agonists and antagonists of the invention may be used to stimulate the regeneration of mucosal surfaces.

[0543] In a specific embodiment, polynucleotides or polypeptides, and/or agonists thereof are used to treat or prevent a disorder characterized by primary or acquired immunodeficiency, deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, polynucleotides or polypeptides, and/or agonists thereof may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carinii. Other diseases and disorders that may be prevented, diagnosed or treated with polynucleotides or polypeptides, and/or agonists of the present invention include, but are not limited to, HIV infection, HTLV-BLV infection, lymphopenia, phagocyte bactericidal dysfunction, anemia, thrombocytopenia, and hemoglobinuria.

[0544] In another embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention are used to treat, and/or diagnose an individual having common variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease.

[0545] In a specific embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to treat, diagnose, and/or prevent cancers or neoplasms including autoimmune cell or tissue-related cancers or neoplasms. Examples of cancers or neoplasms that may be prevented, diagnosed, or treated by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention are described herein and include acute myelogenous leukemia, chronic myelogenous leukemia, Hodgkins disease, non-Hodgkins lymphoma, acute lymphocytic anemia (ALL) Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, and EBV-transformed diseases. In a preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat, diagnose, and/or prevent cancers and neoplasms. In a further preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat, diagnose, and/or prevent, acute myelogenous leukemia.

[0546] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

[0547] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

[0548] In specific embodiments, the compositions of the invention are used as an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete splenectomy.

[0549] Antagonists of the invention include, for example, binding and/or inhibitory antibodies, antisense nucleic acids, ribozymes or soluble forms of the polypeptides of the present invention (e.g., Fc fusion protein) (see e.g., Example 9). Agonists of the invention

include, for example, binding or stimulatory antibodies, and soluble forms of the polypeptides (e.g., Fc fusion proteins) (see e.g., Example 9). polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

[0550] In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are administered to an animal (including, but not limited to, those listed above, and also including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741. Administration of polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention to such animals is useful for the generation of monoclonal antibodies against the polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention.

[0551] Additionally, polynucleotides, polypeptides, and/or antagonists of the invention may affect apoptosis, and therefore, would be useful in treating a number of diseases associated with increased cell survival or the inhibition of apoptosis. For example, diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection.

[0552] In preferred embodiments, polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above.

[0553] Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, emangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

[0554] Diseases associated with increased apoptosis that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid



arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

[0555] Hyperproliferative diseases and/or disorders that could be detected and/or treated by polynucleotides, polypeptides, and/or antagonists of the invention, include, but are not limited to neoplasms located in the: liver, abdomen, bone, breast, digestive system, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

[0556] Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstrom's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

### **Hyperproliferative Disorders**

[0557] CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, can be used to treat or detect hyperproliferative disorders, including neoplasms. CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, may proliferate other cells which can inhibit the hyperproliferative disorder.

[0558] For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

[0559] Examples of hyperproliferative disorders that can be treated or detected by CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

[0560] Similarly, other hyperproliferative disorders can also be treated or detected by CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstrom's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

#### **Cardiovascular Disorders**

[0561] CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, encoding CRCGCL may be used to treat cardiovascular disorders, including peripheral artery disease, such as limb ischemia.

[0562] Cardiovascular disorders include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogly of Fallot, ventricular heart septal defects.

[0563] Cardiovascular disorders also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-

infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

[0564] Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

[0565] Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

[0566] Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

[0567] Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

[0568] Cardiovascular diseases also include vascular diseases such as aneurysms, angiodysplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive

disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, ataxia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

[0569] Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

[0570] Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

[0571] Cerebrovascular disorders include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

[0572] Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

[0573] Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

[0574] CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, are especially effective for the treatment of critical limb ischemia and coronary

disease. As shown in the Examples, administration of CRCGCL polynucleotides and polypeptides to an experimentally induced ischemia rabbit hindlimb may restore blood pressure ratio, blood flow, angiographic score, and capillary density.

[0575] CRCGCL polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. CRCGCL polypeptides may be administered as part of a pharmaceutical composition, described in more detail below. Methods of delivering CRCGCL polynucleotides are described in more detail herein.

#### **Anti-Angiogenesis Activity**

[0576] The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

[0577] The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of the CRCGCL polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of CRCGCL. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)):

[0578] Ocular disorders associated with neovascularization which can be treated with the CRCGCL polynucleotides and polypeptides of the present invention (including CRCGCL agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity, macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

[0579] Additionally, disorders which can be treated with the CRCGCL polynucleotides and polypeptides of the present invention (including CRCGCL agonist and/or antagonists) include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

[0580] Moreover, disorders and/or states, which can be treated with be treated with the CRCGCL polynucleotides and polypeptides of the present invention (including CRCGCL agonist and/or antagonists) include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uvietis, delayed wound healing, endometriosis, vascluogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis,

Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (*Rochela minalia quintosa*), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

#### **Diseases at the Cellular Level**

[0581] Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by CRCGCL polynucleotides or polypeptides, as well as antagonists or agonists of CRCGCL, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, CRCGCL polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

[0582] Additional diseases or conditions associated with increased cell survival that could be treated or detected by CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not

limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

[0583] Diseases associated with increased apoptosis that could be treated or detected by CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

#### **Wound Healing and Epithelial Cell Proliferation**

[0584] In accordance with yet a further aspect of the present invention, there is provided a process for utilizing CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. CRCGCL polynucleotides



or polypeptides, as well as agonists or antagonists of CRCGCL, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could be used to promote dermal reestablishment subsequent to dermal loss

[0585] CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that CRCGCL polynucleotides or polypeptides, agonists or antagonists of CRCGCL, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avacular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omentum graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, can be used to promote skin strength and to improve the appearance of aged skin.

[0586] It is believed that CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. CRCGCL polynucleotides or polypeptides, agonists or antagonists of CRCGCL, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

[0587] CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could also be used to reduce the side effects of gut toxicity that result from

radiation, chemotherapy treatments or viral infections. CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, may have a cytoprotective effect on the small intestine mucosa. CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

[0588] CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with CRCGCL polynucleotides or polypeptides, agonists or antagonists of CRCGCL, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could be used to treat diseases associated with the under expression of CRCGCL.

[0589] Moreover, CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could be used to prevent and heal damage to the lungs due to various pathological states. A growth factor such as CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, which could stimulate proliferation and differentiation and promote the repair of alveoli and bronchiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in

the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using CRCGCL polynucleotides or polypeptides, agonists or antagonists of CRCGCL. Also, CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary dysplasia, in premature infants.

[0590] CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetrachloride and other hepatotoxins known in the art).

[0591] In addition, CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could be used to treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, CRCGCL polynucleotides or polypeptides, as well as agonists or antagonists of CRCGCL, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

### **Infectious Disease**

[0592] CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

[0593] Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL. Examples of viruses, include, but are not limited to the following DNA and RNA viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza), Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. CRCGCL polynucleotides, polypeptides, antibodies and/or agonists or antagonists of the present invention, can be used to treat, prevent, diagnose and/or detect any of these symptoms or diseases.

[0594] Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide, polypeptide, antibody, and/or agonist or antagonist of the present invention include, but are not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), *Cryptococcus neoformans*, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia (e.g., *Borrelia burgdorferi*), Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, *E. coli* (e.g., Enterotoxigenic *E. coli* and Enterohemorrhagic *E. coli*), Enterobacteriaceae (Klebsiella, Salmonella (e.g., *Salmonella typhi*, and *Salmonella paratyphi*), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, *Mycobacterium leprae*, *Vibrio cholerae*, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Meningococcal), *Meisseria meningitidis*, Pasteurellaceae Infections (e.g., Actinobacillus, Haemophilus (e.g., *Haemophilus influenza* type B), Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Treponema

spp., *Leptospira* spp., *Shigella* spp., Staphylococcal, Meningococcal, Pneumococcal and Streptococcal (e.g., *Streptococcus pneumoniae* and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., meningitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. CRCGCL polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases.

[0595] Moreover, parasitic agents causing disease or symptoms that can be treated or detected by CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, include, but not limited to, the following families: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas. These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), Malaria, pregnancy complications, and toxoplasmosis. CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, can be used to treat or detect any of these symptoms or diseases.

[0596] Preferably, treatment using CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, could either be by administering an effective amount of CRCGCL polypeptide to the patient, or by removing cells from the patient, supplying the cells with CRCGCL polynucleotide, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the CRCGCL polypeptide or polynucleotide can be used as an antigen in a vaccine to raise an immune response against infectious disease.

## **Regeneration**

[0597] CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

[0598] Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

[0599] Moreover, CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

[0600] Similarly, nerve and brain tissue could also be regenerated by using CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL.

### **Chemotaxis**

[0601] CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

[0602] CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. As a chemotactic molecule, CRCGCL could also attract fibroblasts, which can be used to treat wounds.

[0603] It is also contemplated that CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, could be used as an inhibitor of chemotaxis.

### **Binding Activity**

[0604] CRCGCL polypeptides may be used to screen for molecules that bind to CRCGCL or for molecules to which CRCGCL binds. The binding of CRCGCL and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the CRCGCL or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

[0605] Preferably, the molecule is closely related to the natural ligand of CRCGCL, e.g., TSLP, a fragment of TSLP, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) In either case, the molecule can be rationally designed using known techniques.

[0606] Preferably, the screening for these molecules involves producing appropriate cells which express CRCGCL, either as a secreted protein or on the cell membrane.

Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing CRCGCL (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either CRCGCL or the molecule.

[0607] The assay may simply test binding of a candidate compound to CRCGCL, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to CRCGCL.

[0608] Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing CRCGCL, measuring CRCGCL/molecule activity or binding, and comparing the CRCGCL/molecule activity or binding to a standard.

[0609] Preferably, an ELISA assay can measure CRCGCL level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure CRCGCL level or activity by either binding, directly or indirectly, to CRCGCL or by competing with CRCGCL for a substrate (e.g., TSLP).

[0610] Additionally, the receptor to which CRCGCL binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., *Current Protocols in Immun.*, 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

[0611] Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.



[0612] As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

[0613] Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of CRCGCL thereby effectively generating agonists and antagonists of CRCGCL. See generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., *et al.*, *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., *et al.*, *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of CRCGCL polynucleotides and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired CRCGCL molecule by homologous, or site-specific, recombination. In another embodiment, CRCGCL polynucleotides and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of CRCGCL may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are Cytokine Receptor family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

[0614] Other preferred fragments are biologically active CRCGCL fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the CRCGCL polypeptide. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

[0615] Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and  $^3\text{[H]}$  thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of  $^3\text{[H]}$  thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of  $^3\text{[H]}$  thymidine. Both agonist and antagonist compounds may be identified by this procedure.

[0616] In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the CRCGCL receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

[0617] All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the CRCGCL/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of CRCGCL from suitably manipulated cells or tissues. Therefore, the invention includes a method of identifying compounds which bind to CRCGCL comprising the steps of: (a) incubating a candidate binding compound with CRCGCL; and (b) determining if binding has occurred. Moreover, the invention includes a method of

identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with CRCGCL, (b) assaying a biological activity, and (b) determining if a biological activity of CRCGCL has been altered.

[0618] Also, one could identify molecules bind CRCGCL experimentally by using the beta-pleated sheet regions disclosed in Figure 3 and Table 1. Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of each beta pleated sheet regions disclosed in Figure 3/Table 1. Additional embodiments of the invention are directed to polynucleotides encoding CRCGCL polypeptides which comprise, or alternatively consist of, any combination or all of the beta pleated sheet regions disclosed in Figure 3/Table 1. Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the CRCGCL amino acid sequence of each of the beta pleated sheet regions disclosed in Figure 3/Table 1. Additional embodiments of the invention are directed to CRCGCL polypeptides which comprise, or alternatively consist of, any combination or all of the beta pleated sheet regions disclosed in Figure 3/Table 1.

#### **Targeted Delivery**

[0619] In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

[0620] As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

[0621] In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

[0622] In another embodiment, the invention provides a method for the specific

destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

[0623] By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

### **Drug Screening**

[0624] Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

[0625] This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or

fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

[0626] Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

[0627] Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

[0628] This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

#### **Antisense And Ribozyme (Antagonists)**

[0629] In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:1, or the

complementary strand thereof, and/or to nucleotide sequences contained in the deposited clone 209641 or 209691. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, J., *Neurochem.* 56:560 (1991). *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J., *Neurochem.* 56:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., *Nucleic Acids Research* 6:3073 (1979); Cooney et al., *Science* 241:456 (1988); and Dervan et al., *Science* 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

[0630] For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

[0631] In one embodiment, the CRCGCL antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the CRCGCL antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding CRCGCL, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature* 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797 (1980), the herpes

thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., Nature 296:39-42 (1982)), etc.

[0632] The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a CRCGCL gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded CRCGCL antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a CRCGCL RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[0633] Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, Nature 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions of CRCGCL shown in Figures 1A-B could be used in an antisense approach to inhibit translation of endogenous CRCGCL mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of CRCGCL mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

[0634] The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The

oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre et al., 1987, *Proc. Natl. Acad. Sci.* 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, e.g., Zon, 1988, *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

[0635] The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

[0636] The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

[0637] In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.



[0638] In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

[0639] Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

[0640] While antisense nucleotides complementary to the CRCGCL coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

[0641] Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy CRCGCL mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of CRCGCL (Figures 1A-B). Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the CRCGCL mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

[0642] As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express CRCGCL in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of

antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous CRCGCL messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

[0643] Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

[0644] The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirable in cases such as restenosis after balloon angioplasty.

[0645] The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

[0646] The antagonist/agonist may also be employed to treat the diseases described herein.

#### **Binding Peptides and Other Molecules**

[0647] The invention also encompasses screening methods for identifying polypeptides and nonpolypeptides that bind CRCGCL polypeptides, and the CRCGCL binding molecules identified thereby. These binding molecules are useful, for example, as agonists and antagonists of CRCGCL polypeptides. Such agonists and antagonists can be used, in accordance with the invention, in the therapeutic embodiments described in detail, below.

[0648] This method comprises the steps of:

a) contacting CRCGCL polypeptides or CRCGCL-like polypeptides with a plurality of molecules; and

b) identifying a molecule that binds the CRCGCL polypeptides or CRCGCL-like polypeptides.

[0649] The step of contacting CRCGCL polypeptides or CRCGCL-like polypeptides

with the plurality of molecules may be effected in a number of ways. For example, one may contemplate immobilizing CRCGCL polypeptides or CRCGCL-like polypeptides on a solid support and bringing a solution of the plurality of molecules in contact with the immobilized CRCGCL polypeptides or CRCGCL-like polypeptides. Such a procedure would be akin to an affinity chromatographic process, with the affinity matrix being comprised of the immobilized CRCGCL polypeptides or CRCGCL-like polypeptides. The molecules having a selective affinity for the CRCGCL polypeptides or CRCGCL-like polypeptides can then be purified by affinity selection. The nature of the solid support, process for attachment of the CRCGCL polypeptides or CRCGCL-like polypeptides to the solid support, solvent, and conditions of the affinity isolation or selection are largely conventional and well known to those of ordinary skill in the art.

[0650] Alternatively, one may also separate a plurality of polypeptides into substantially separate fractions comprising a subset of or individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or like method known to those of ordinary skill for the separation of polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be expressed on or about its outer surface (e.g., a recombinant phage). Individual isolates can then be "probed" by the CRCGCL polypeptides or CRCGCL-like polypeptides, optionally in the presence of an inducer should one be required for expression, to determine if any selective affinity interaction takes place between the CRCGCL polypeptides or CRCGCL-like polypeptides and the individual clone. Prior to contacting the CRCGCL polypeptides or CRCGCL-like polypeptides with each fraction comprising individual polypeptides, the polypeptides could first be transferred to a solid support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon. In this manner, positive clones could be identified from a collection of transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for CRCGCL polypeptides or CRCGCL-like polypeptides. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for the CRCGCL polypeptides or CRCGCL-like polypeptides can be determined directly by conventional means or the coding sequence of the DNA encoding the polypeptide can frequently be determined more conveniently. The primary sequence can then be deduced from the corresponding DNA sequence. If the amino acid sequence is to be

determined from the polypeptide itself, one may use microsequencing techniques. The sequencing technique may include mass spectroscopy.

[0651] In certain situations, it may be desirable to wash away any unbound CRCGCL polypeptides or CRCGCL-like polypeptides, or alternatively, unbound polypeptides, from a mixture of the CRCGCL polypeptides or CRCGCL-like polypeptides and the plurality of polypeptides prior to attempting to determine or to detect the presence of a selective affinity interaction. Such a wash step may be particularly desirable when the CRCGCL polypeptides or CRCGCL-like polypeptides or the plurality of polypeptides is bound to a solid support.

[0652] The plurality of molecules provided according to this method may be provided by way of diversity libraries, such as random or combinatorial peptide or nonpeptide libraries which can be screened for molecules that specifically bind CRCGCL polypeptides. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant (e.g., phage display libraries), and in vitro translation-based libraries. Examples of chemically synthesized libraries are described in Fodor et al., 1991, *Science* 251:767-773; Houghten et al., 1991, *Nature* 354:84-86; Lam et al., 1991, *Nature* 354:82-84; Medynski, 1994, *Bio/Technology* 12:709-710; Gallop et al., 1994, *J. Medicinal Chemistry* 37(9):1233-1251; Ohlmeyer et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:10922-10926; Erb et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:11422-11426; Houghten et al., 1992, *Biotechniques* 13:412; Jayawickreme et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:1614-1618; Salmon et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:11708-11712; PCT Publication No. WO 93/20242; and Brenner and Lerner, 1992, *Proc. Natl. Acad. Sci. USA* 89:5381-5383.

[0653] Examples of phage display libraries are described in Scott and Smith, 1990, *Science* 249:386-390; Devlin et al., 1990, *Science*, 249:404-406; Christian, R. B., et al., 1992, *J. Mol. Biol.* 227:711-718; Lenstra, 1992, *J. Immunol. Meth.* 152:149-157; Kay et al., 1993, *Gene* 128:59-65; and PCT Publication No. WO 94/18318 dated Aug. 18, 1994.

[0654] In vitro translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058 dated Apr. 18, 1991; and Mattheakis et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:9022-9026.

[0655] By way of examples of nonpeptide libraries, a benzodiazepine library (see e.g., Bunin et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in

peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (1994, Proc. Natl. Acad. Sci. USA 91:11138-11142).

[0656] The variety of non-peptide libraries that are useful in the present invention is great. For example, Ecker and Crooke, 1995, Bio/Technology 13:351-360 list benzodiazepines, hydantoins, piperazinediones, biphenyls, sugar analogs, beta-mercaptoketones, arylacetic acids, acylpiperidines, benzopyrans, cubanes, xanthines, aminimides, and oxazolones as among the chemical species that form the basis of various libraries.

[0657] Non-peptide libraries can be classified broadly into two types: decorated monomers and oligomers. Decorated monomer libraries employ a relatively simple scaffold structure upon which a variety functional groups is added. Often the scaffold will be a molecule with a known useful pharmacological activity. For example, the scaffold might be the benzodiazepine structure.

[0658] Non-peptide oligomer libraries utilize a large number of monomers that are assembled together in ways that create new shapes that depend on the order of the monomers. Among the monomer units that have been used are carbamates, pyrrolinones, and morpholinos. Peptoids, peptide-like oligomers in which the side chain is attached to the alpha amino group rather than the alpha carbon, form the basis of another version of non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single type of monomer and thus contained a repeating backbone. Recent libraries have utilized more than one monomer, giving the libraries added flexibility.

[0659] Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, 1989, Adv. Exp. Med. Biol. 251:215-218; Scott and Smith, 1990, Science 249:386-390; Fowlkes et al., 1992; BioTechniques 13:422-427; Oldenburg et al., 1992, Proc. Natl. Acad. Sci. USA 89:5393-5397; Yu et al., 1994, Cell 76:933-945; Staudt et al., 1988, Science 241:577-580; Bock et al., 1992, Nature 355:564-566; Tuerk et al., 1992, Proc. Natl. Acad. Sci. USA 89:6988-6992; Ellington et al., 1992, Nature 355:850-852; U.S. Pat. No. 5,096,815, U.S. Pat. No. 5,223,409, and U.S. Pat. No. 5,198,346, all to Ladner et al.; Rebar and Pabo, 1993, Science 263:671-673; and CT Publication No. WO 94/18318.

[0660] In a specific embodiment, screening to identify a molecule that binds CRCGCL polypeptides can be carried out by contacting the library members with a

CRCGCL polypeptides or CRCGCL-like polypeptides immobilized on a solid phase and harvesting those library members that bind to the CRCGCL polypeptides or CRCGCL-like polypeptides. Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley and Smith, 1988, Gene 73:305-318; Fowlkes et al., 1992, BioTechniques 13:422-427; PCT Publication No. WO 94/18318; and in references cited herein.

[0661] In another embodiment, the two-hybrid system for selecting interacting proteins in yeast (Fields and Song, 1989, Nature 340:245-246; Chien et al., 1991, Proc. Natl. Acad. Sci. USA 88:9578-9582) can be used to identify molecules that specifically bind to CRCGCL polypeptides or CRCGCL-like polypeptides.

[0662] Where the CRCGCL binding molecule is a polypeptide, the polypeptide can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term "biased" is used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that govern the diversity of the resulting collection of molecules, in this case peptides.

[0663] Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is the same for all 20 amino acids. A bias can be introduced into the library, however, by specifying, for example, that a lysine occur every fifth amino acid or that positions 4, 8, and 9 of a decapeptide library be fixed to include only arginine. Clearly, many types of biases can be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates specific types of peptide libraries, such as phage displayed peptide libraries and those that utilize a DNA construct comprising a lambda phage vector with a DNA insert.

[0664] As mentioned above, in the case of a CRCGCL binding molecule that is a polypeptide, the polypeptide may have about 6 to less than about 60 amino acid residues, preferably about 6 to about 10 amino acid residues, and most preferably, about 6 to about 22 amino acids. In another embodiment, a CRCGCL binding polypeptide has in the range of 15-100 amino acids, or 20-50 amino acids.

[0665] The selected CRCGCL binding polypeptide can be obtained by chemical synthesis or recombinant expression.

### **Other Activities**

[0666] The polypeptide of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. These polypeptide may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

[0667] The polypeptide may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

[0668] The polypeptide of the present invention may also be employed stimulate neuronal growth and to treat and prevent neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. CRCGCL may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

[0669] The polypeptide of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

[0670] The CRCGCL polypeptide may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, the polypeptides of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

[0671] The CRCGCL polypeptide may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

[0672] The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

[0673] CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

[0674] CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

[0675] CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, may be used to change a mammal's mental state or physical state by influencing biorhythms, cardiac rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

[0676] CRCGCL polynucleotides or polypeptides, or agonists or antagonists of CRCGCL, may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

[0677] The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

[0678] Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

[0679] Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

## Examples



*Example 1: Isolation of the CRCGCL cDNA Clone  
From the Deposited Sample*

[0680] The cDNA for CRCGCL is inserted into the EcoRI/XhoI multiple cloning site of Uni-ZAP XR (Stratagene). Uni-ZAP XR contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59- (1993).)

[0681] Two approaches can be used to isolate CRCGCL from the deposited sample. First, the deposited clone is transformed into a suitable host (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. A single colony is then used to generate DNA using nucleic acid isolation techniques well known to those skilled in the art. (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press.)

[0682] Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:1 (i.e., within the region of SEQ ID NO:1 bounded by the 5' NT and the 3' NT of the clone) are synthesized and used to amplify the CRCGCL cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 ul of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is 1.5-5 mM MgCl<sub>2</sub>, 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

[0683] Several methods are available for the identification of the 5' or 3' non-coding portions of the CRCGCL gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes,

and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

[0684] Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the CRCGCL gene of interest is used to PCR amplify the 5' portion of the CRCGCL full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

[0685] This above method starts with total RNA isolated from the desired source, although poly-A<sup>+</sup> RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

[0686] This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the CRCGCL gene.

### *Example 2: Isolation of CRCGCL Genomic Clones*

[0687] A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:1., according to the method described in Example 1. (See also, Sambrook.)

### *Example 3: Tissue Distribution of CRCGCL Polypeptides*

[0688] Tissue distribution of mRNA expression of CRCGCL is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a CRCGCL probe produced by the method described in Example 1 is labeled with  $P^{32}$  using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

[0689] Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 degree C overnight, and the films developed according to standard procedures.

### *Example 4: Chromosomal Mapping of CRCGCL*

[0690] An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:1. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

### *Example 5: Bacterial Expression of CRCGCL*

[0691] CRCGCL polynucleotide encoding a CRCGCL polypeptide invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to

amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

[0692] Specifically, to clone the CRCGCL protein in a bacterial vector, the 5' primer has the sequence 5' GTTAGGCCATGGGAGGAGCAGCAGAAGGA 3' (SEQ ID NO: 14) containing the Nco I restriction site followed a number of nucleotides of the amino terminal coding sequence of the CRCGCL sequence in SEQ ID NO:1. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete CRCGCL protein shorter or longer than the the portion described above. The 3' primer has the sequence 5' GGTTAAAGATCTCAACGCCACGTAGGAGCGGTC 3' (SEQ ID NO: 15) containing the BglII restriction site followed by a number nucleotides complementary to the 3' end of the coding sequence of the CRCGCL DNA sequence of SEQ ID NO:1.

[0693] The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

[0694] Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalactopyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

[0695] Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilotri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., supra). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., supra).

[0696] Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

[0697] The purified CRCGCL protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the CRCGCL protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified CRCGCL protein is stored at 4 degree C or frozen at -80 degree C.

[0698] In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a CRCGCL polynucleotide, called pHE4a. (ATCC Accession Number 209645, deposited February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The origin of replication (oriC) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

[0699] DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the

larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

[0700] The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system. More preferably, the bacterial expression vector, pQE60 can also be used to express CRCGCL.

#### *Example 6: Purification of CRCGCL Polypeptide from an Inclusion Body*

[0701] The following alternative method can be used to purify CRCGCL polypeptide expressed in E coli when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

[0702] Upon completion of the production phase of the E. coli fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

[0703] The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

[0704] The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

[0705] Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20

volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

[0706] To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16  $\mu$ m membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

[0707] Fractions containing the CRCGCL polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

[0708] The resultant CRCGCL polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5  $\mu$ g of purified protein is loaded. The purified CRCGCL protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

#### *Example 7: Cloning and Expression of CRCGCL in a Baculovirus Expression System*

[0709] In this example, the plasmid shuttle vector pA2 is used to insert CRCGCL polynucleotide into a baculovirus to express CRCGCL. This expression vector contains the

strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned CRCGCL polynucleotide.

[0710] Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

[0711] Specifically, the CRCGCL cDNA sequence contained in the deposited clone, including the AUG initiation codon and any naturally associated leader sequence, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

[0712] More specifically, the cDNA sequence encoding the full length CRCGCL protein in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence shown in SEQ ID NO:1, is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. For example, the 5' primer could have the sequence 5' CCGGTTAGATCTGCCATCATGGCTTTGGGGCAAGGAGG 3' (SEQ ID NO: 16) containing the BglII restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells (Kozak, M., *J. Mol. Biol.* 196:947-950 (1987)), followed by a number of nucleotides of the sequence of the complete CRCGCL protein shown in Figures 1A-1B. Alternatively, the 5' primer: 5' CCGGTTAGATCTGCCATCATGGGGCGGCTGGTTCTG 3' (SEQ ID NO:28), also having a Bgl II restriction site could also be used. The 3' primer has the sequence 5'



CCGGTTTCTAGATCACAACGCCACGTAGGAGCGGTC 3' (SEQ ID NO: 17)  
containing the XbaI restriction site followed by a number of nucleotides complementary to the 3' noncoding sequence in Figures 1A-1B.

[0713] The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

[0714] The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

[0715] The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

[0716] Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGold™ virus DNA and 5 ug of the plasmid are mixed in a sterile well of a microtiter plate containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

[0717] After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, supra. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque

assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

[0718] To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of  $^{35}\text{S}$ -methionine and 5 uCi  $^{35}\text{S}$ -cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

[0719] Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced CRCGCL protein.

### *.. Example 8: Expression of CRCGCL in Mammalian Cells*

[0720] CRCGCL polypeptide can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

[0721] Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2DHFR (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

[0722] Alternatively, CRCGCL polypeptide can be expressed in stable cell lines containing the CRCGCL polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as DHFR, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

[0723] The transfected CRCGCL gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

[0724] Derivatives of the plasmid pSV2-DHFR (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of CRCGCL. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

[0725] Specifically, the plasmid pC6 or pC4 is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in

the art. The vector is then isolated from a 1% agarose gel. Also preferred is the pcDNA3 vector (Life Technologies).

[0726] If a naturally occurring signal sequence is used to produce a secreted protein, the vector does not need a second signal peptide. Alternatively, if a naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence in an effort to secrete the protein from the cell. (See, e.g., WO 96/34891.)

[0727] The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 or pC4 using, for instance, restriction enzyme analysis.

[0728] For example, a soluble CRCGCL polypeptide, such as amino acids Met 1 to Lys 231, could also be expressed. A 5' primer: 5' CCGGTTAGATCTGCCATCATGGGGCGGCTGGTTCTG 3' (SEQ ID NO:28), having a Bgl II restriction site and a 3' primer: 5' GGCCGGTCTAGATTATTTGGACAGCTTTGGTTTG 3' (SEQ ID NO:31) could be used to PCR amino acids Met 1 to Lys 231. The amplified product could be inserted into a mammalian expression vector, such as pC4 or pC6.

[0729] Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five µg of the expression plasmid pC6 or pC4 is cotransfected with 0.5 µg of the plasmid pSVneo using lipofectin (Felgner et al., supra). The plasmid pSV2-neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 µM, 2 µM, 5 µM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a

concentration of 100 - 200 uM. Expression of CRCGCL is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

*Example 9: Construction of N-Terminal and/or  
C-Terminal Deletion Mutants*

[0730] The following general approach may be used to clone a N-terminal or C-terminal deletion CRCGCL deletion mutant. Generally, two oligonucleotide primers of about 15-25 nucleotides are derived from the desired 5' and 3' positions of a polynucleotide of SEQ ID NO:1. The 5' and 3' positions of the primers are determined based on the desired CRCGCL polynucleotide fragment. An initiation and stop codon are added to the 5' and 3' primers respectively, if necessary, to express the CRCGCL polypeptide fragment encoded by the polynucleotide fragment. Preferred CRCGCL polynucleotide fragments are those encoding the N-terminal and C-terminal deletion mutants disclosed above in the "Polynucleotide and Polypeptide Fragments" section of the Specification.

[0731] Additional nucleotides containing restriction sites to facilitate cloning of the CRCGCL polynucleotide fragment in a desired vector may also be added to the 5' and 3' primer sequences. The CRCGCL polynucleotide fragment is amplified from genomic DNA or from the deposited cDNA clone using the appropriate PCR oligonucleotide primers and conditions discussed herein or known in the art. The CRCGCL polypeptide fragments encoded by the CRCGCL polynucleotide fragments of the present invention may be expressed and purified in the same general manner as the full length polypeptides, although routine modifications may be necessary due to the differences in chemical and physical properties between a particular fragment and full length polypeptide.

[0732] As a means of exemplifying but not limiting the present invention, the polynucleotide encoding the CRCGCL polypeptide fragment I-35 to F-276 is amplified and cloned as follows: A 5' primer is generated comprising a restriction enzyme site followed by an initiation codon in frame with the polynucleotide sequence encoding the N-terminal portion of the polypeptide fragment beginning with I-35. A complementary 3' primer is generated comprising a restriction enzyme site followed by a stop codon in frame with the polynucleotide sequence encoding C-terminal portion of the CRCGCL polypeptide fragment ending with F-276.

[0733] The amplified polynucleotide fragment and the expression vector are digested with restriction enzymes which recognize the sites in the primers. The digested polynucleotides are then ligated together. The CRCGCL polynucleotide fragment is inserted into the restricted expression vector, preferably in a manner which places the CRCGCL polypeptide fragment coding region downstream from the promoter. The ligation mixture is transformed into competent *E. coli* cells using standard procedures and as described in the Examples herein. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

#### *Example 10: Protein Fusions of CRCGCL*

[0734] CRCGCL polypeptides are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of CRCGCL polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to CRCGCL polypeptides can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

[0735] Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

[0736] For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and CRCGCL polynucleotide, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site.

[0737] Alternatively, a soluble CRCGCL polypeptide, such as amino acids Met 1 to Lys 231, could also be fused to the Fc portion. For Example, a 5' primer: 5' CCGGTTAGATCTGCCATCATGGGGCGGCTGGTTCTG 3' (SEQ ID NO:28), having a Bgl II restriction site and a 3' primer: 5' GGCCGGTCTAGATTTGGACAGCTTTGGTTTG 3' (SEQ ID NO:29) could be used to PCR amino acids Met 1 to Lys 231. The amplified product could be fused to Fc to produce a Fc fusion protein, as set forth above, and ligated to the pC4 vector for mammalian expression or pA2 for baculovirus expression.

[0738] In either case, note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced. Moreover, if the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

[0739] The Fc fusions described above could also be inserted into the pA2 vector to express in Baculovirus systems, as set forth in Example 7, using techniques known in the art and described herein.

#### Human IgG Fc region:

```
GGGATCCGGAGCCCAAATCTTCTGACAAAACCTCACACATGCCACCGTGCCACGACCTGAAT
TCGAGGGTGCAACCGTCAGTCTTCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCG
GACTCCTGAGGTGCACATGCGTGGTGGTGACGTAAGCCACGAAGACCGTGAGGTCAAGTTCA
ACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC
AACAGCACGTACCGTGTGGTCAGCGTCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAG
GAGTAÇAAGTGCAAGGTCTCCAACAAAGCCCTCCCAACCCCCATCGAGAAAAACCATCTCCAA
AGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGA
CCAAGAACCAGGTCAACCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGTGG
AGTGGGAGAGCAATGGGCAGCCGGAGAACAATAAGACACGCTCCCGTGCTGGACTCC
GACGGCTCCTTCTTCTCTACAGCAAGCTACCGTGGACAAGAGCAGGTGGCAGCAGGGGAA
CGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTC
CCTGTCTCCGGGTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:4)
```

### *Example 11: Production of an Antibody*

#### Hybridoma Technology

[0740] The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells

expressing CRCGCL is administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of CRCGCL protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

[0741] In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., *Nature* 256:495 (1975); Köhler et al., *Eur. J. Immunol.* 6:511 (1976); Köhler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with CRCGCL polypeptide or, more preferably, with a secreted CRCGCL polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degree C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

[0742] The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology* 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the CRCGCL polypeptide.

[0743] Alternatively, additional antibodies capable of binding to CRCGCL polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the CRCGCL protein-specific antibody can be blocked by CRCGCL. Such antibodies comprise



anti-idiotypic antibodies to the CRCGCL protein-specific antibody and can be used to immunize an animal to induce formation of further CRCGCL protein-specific antibodies.

[0744] It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, secreted CRCGCL protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

[0745] For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

#### **Isolation of antibody fragments directed against CRCGCL from a library of scFvs.**

[0746] Naturally occurring V-genes isolated from human PBLs are constructed into a large library of antibody fragments which contain reactivities against CRCGCL to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein in its entirety by reference).

[0747] *Rescue of the Library.* A library of scFvs is constructed from the RNA of human PBLs as described in WO92/01047. To rescue phage displaying antibody fragments, approximately 10<sup>9</sup> E. coli harbouring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 ug/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10<sup>8</sup> TU of delta gene 3 helper (M13 delta gene III, see WO92/01047) are added and the culture incubated at 37 degree C for 45 minutes without shaking and then at 37 degree C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 ug/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in WO92/01047.

[0748] M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harbouring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37 degree C without shaking and then for a further hour at 37 degree C with shaking. Cells are spun down (IEC-Centra 8, 4000 revs/min for 10 min), resuspended in 300 ml 2xTY broth containing 100 ug ampicillin/ml and 25 ug kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37° C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 um filter (Minisart NML; Sartorius) to give a final concentration of approximately  $10^{13}$  transducing units/ml (ampicillin-resistant clones).

[0749] *Panning of the Library.* Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 ug/ml or 10 ug/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37 degree C and then washed 3 times in PBS. Approximately  $10^{13}$  TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37 degree C. The E. coli are then plated on TYE plates containing 1% glucose and 100 ug/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

[0750] *Characterization of Binders.* Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH

9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see e.g., WO92/01047) and then by sequencing.

*Example 12: Production Of CRCGCL Protein For  
High-Throughput Screening Assays*

[0751] The following protocol produces a supernatant containing CRCGCL polypeptide to be tested. This supernatant can then be used in the Screening Assays described in Examples 14-21.

[0752] First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

[0753] Plate 293T cells (do not carry cells past P+20) at  $2 \times 10^5$  cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

[0754] The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8-10, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

[0755] Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time

on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Cotimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degree C for 6 hours.

[0756] While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or HGS CHO-5 media (116.6 mg/L of CaCl<sub>2</sub> (anhyd); 0.00130 mg/L CuSO<sub>4</sub>·5H<sub>2</sub>O; 0.050 mg/L of Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O; 0.417 mg/L of FeSO<sub>4</sub>·7H<sub>2</sub>O; 311.80 mg/L of KCl; 28.64 mg/L of MgCl<sub>2</sub>; 48.84 mg/L of MgSO<sub>4</sub>; 6995.50 mg/L of NaCl; 2400.0 mg/L of NaHCO<sub>3</sub>; 62.50 mg/L of NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O; 71.02 mg/L of Na<sub>2</sub>HPO<sub>4</sub>; 4320 mg/L of ZnSO<sub>4</sub>·7H<sub>2</sub>O; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitic Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine-H<sub>2</sub>O; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H<sub>2</sub>O; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L- Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L- Histidine-HCL-H<sub>2</sub>O; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2H<sub>2</sub>O; and 99.65 mg/ml of L- Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; 0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal Acetate. Adjust

osmolarity to 327 mOsm) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

[0757] The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degree C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

[0758] On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 14-21.

[0759] It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the CRCGCL polypeptide directly (e.g., as a secreted protein) or by CRCGCL inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

### *Example 13: Construction of GAS Reporter Construct*

[0760] One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

[0761] GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. STAT5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

[0762] The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

[0763] The Jaks are activated by a wide range of receptors summarized in Table 2 below. (Adapted from review by Schidler and Darnell, *Ann. Rev. Biochem.* 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN- $\alpha$ , IFN- $\gamma$ , and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:5)).

[0764] Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

[0765] Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table 2 below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

[0766] .. There is preliminary data that CRCGCL interacts with Jak1.

**Table 2**

<u>Ligand</u>	<u>tyk2</u>	<u>JAKs</u>				<u>STATS GAS(elements) or ISRE</u>
		<u>Jak1</u>	<u>Jak2</u>	<u>Jak3</u>		
<u>IFN family</u>						
IFN-a/B	+	+	-	-	1,2,3	ISRE
IFN-g		+	+	-	1	GAS (IRF1>Lys6>IFP)
Il-10	+	?	?	-	1,3	
<u>gp130 family</u>						
IL-6 (Pleiotrohic)	+	+	+	?	1,3	GAS (IRF1>Lys6>IFP)
Il-11(Pleiotrohic)	?	+	?	?	1,3	
OnM(Pleiotrohic)	?	+	+	?	1,3	
LIF(Pleiotrohic)	?	+	+	?	1,3	
CNTF(Pleiotrohic)	-/+	+	+	?	1,3	
G-CSF(Pleiotrohic)	?	+	?	?	1,3	
IL-12(Pleiotrohic)	+	-	+	+	1,3	
<u>g-C family</u>						
IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
IL-4 (lymph/myeloid)	-	+	-	+	6	GAS (IRF1=IFP>Ly6)(IgH)
IL-7 (lymphocytes)	-	+	-	+	5	GAS
IL-9 (lymphocytes)	-	+	-	+	5	GAS
IL-13 (lymphocyte)	-	+	?	?	6	GAS
IL-15	?	+	?	+	5	GAS
<u>gp140 family</u>						
IL-3 (myeloid)	-	-	+	-	5	GAS (IRF1>IFP>>Ly6)
IL-5 (myeloid)	-	-	+	-	5	GAS
GM-CSF (myeloid)	-	-	+	-	5	GAS
<u>Growth hormone family</u>						
GH	?	-	+	-	5	
PRL	?	+/-	+	-	1,3,5	
EPO	?	-	+	-	5	GAS(B-
CAS>IRF1=IFP>>Ly6)						
<u>Receptor Tyrosine Kinases</u>						
EGF	?	+	+	-	1,3	GAS (IRF1)
PDGF	?	+	+	-	1,3	
CSF-1	?	+	+	-	1,3	GAS (not IRF1)

[0767] To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 14-15, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., *Immunity* 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is: 5':GCGCCTCGAGATTTCCCGAAATCTAGATTTCCCGAAATGATTTCCCGAAA TGATTTCCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:6).

[0768] The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:7).

[0769] PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence: 5':CTCGAGATTTCCCGAAATCTAGATTTCCCGAAATGATTTCCCGAAATGATT TCCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACT CCGCCCATCCCGCCCCTAACTCCGCCAGTTCCGCCATTCTCCGCCCATGGCT GACTAATTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTC CAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTT:3' (SEQ ID NO:8).

[0770] With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.



[0771] The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

[0772] Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 14-15.

[0773] Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 16 and 17. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

#### *Example 14: High-Throughput Screening Assay for T-cell Activity.*

[0774] The following protocol is used to assess T-cell activity of CRCGCL by determining whether CRCGCL supernatant proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 13. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

[0775] Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

[0776] Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

[0777] During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37 degree C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

[0778] The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with supernatants containing CRCGCL polypeptides or CRCGCL induced polypeptides as produced by the protocol described in Example 12.

[0779] On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

[0780] Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

[0781] After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

[0782] The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophane cover) and stored at -20 degree C until SEAP assays are performed according to Example 18. The plates containing the remaining treated cells are placed at 4 degree C and serve as a source of material for repeating the assay on a specific well if desired.

[0783] As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

#### *Example 15: High-Throughput Screening Assay Identifying Myeloid Activity*

[0784] The following protocol is used to assess myeloid activity of CRCGCL by determining whether CRCGCL proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 13. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

[0785] To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 13, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest  $2 \times 10^7$  U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

[0786] Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 1 mM  $\text{MgCl}_2$ , and 675 uM  $\text{CaCl}_2$ . Incubate at 37 degree C for 45 min.

[0787] Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degree C for 36 hr.

[0788] The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

[0789] These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200 ul cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

[0790] Add 50 ul of the supernatant prepared by the protocol described in Example 12. Incubate at 37 degree C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 18.

#### *Example 16: High-Throughput Screening Assay Identifying Neuronal Activity.*

[0791] When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed by CRCGCL.

[0792] Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat pheochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells by CRCGCL can be assessed.

[0793] The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:9) and 5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:10).

[0794] Using the GAS:SEAP/Neo vector produced in Example 13, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

[0795] To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

[0796] PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

[0797] Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 12. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

[0798] To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

[0799] The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as  $5 \times 10^5$  cells/ml.

[0800] Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to  $1 \times 10^5$  cells/well). Add 50 ul supernatant produced by Example 12, 37 degree C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP

is typically seen in the positive control wells. SEAP assay the supernatant according to Example 18.

### *Example 17: High-Throughput Screening Assay for T-cell Activity*

[0801] NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF-KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

[0802] In non-stimulated conditions, NF-KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I-KB is phosphorylated and degraded, causing NF-KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF-KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

[0803] Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 12. Activators or inhibitors of NF-KB would be useful in treating diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

[0804] To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:11), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site: 5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTC CATCCTGCCATCTCAATTAG:3' (SEQ ID NO:12).

[0805] The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:7).

[0806] PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is

digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene) Sequencing with the T7 and T3 primers confirms the insert contains the following sequence: 5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCCATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTT:3' (SEQ ID NO:13).

[0807] Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

[0808] In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes Sall and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with Sall and NotI.

[0809] Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 14. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 14. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

### *Example 18: Assay for SEAP Activity*

[0810] As a reporter molecule for the assays described in Examples 14-17, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

[0811] Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

**[0812]** Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see Table 3 below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

**[0813]** Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

**Table 3**

**Reaction Buffer Formulation:**

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25
24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8



31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

---

***Example 19: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability***

[0814] Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

[0815] The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-3, used here.

[0816] For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for 20

hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

[0817] A stock solution of 1 mg/ml fluo-3 is made in 10% pluronic acid DMSO. To load the cells with fluo-3, 50 ul of 12 ug/ml fluo-3 is added to each well. The plate is incubated at 37 degree C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

[0818] For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10<sup>6</sup> cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-3 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degree C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10<sup>6</sup> cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 ul, followed by an aspiration step to 100 ul final volume.

[0819] For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-3. The supernatant is added to the well, and a change in fluorescence is detected.

[0820] To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event caused by the a molecule, either CRCGCL or a molecule induced by CRCGCL, which has resulted in an increase in the intracellular Ca<sup>++</sup> concentration.

### *Example 20: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity*

[0821] The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

[0822] Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

[0823] Because of the wide range of known factors capable of stimulating tyrosine kinase activity, identifying whether CRCGCL or a molecule induced by CRCGCL is capable of activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

[0824] Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

[0825] To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 12, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and

the plate is shaken on a rotating shaker for 5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degree C at 16,000 x g.

[0826] Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

[0827] Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

[0828] The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg<sub>2</sub><sup>+</sup> (5mM ATP/50mM MgCl<sub>2</sub>), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degree C for 2 min. Initiate the reaction by adding 10ul of the control enzyme or the filtered supernatant.

[0829] The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

[0830] Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degree C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degree C for one hour. Wash the well as above.

[0831] Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of

the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

*Example 21: High-Throughput Screening Assay  
Identifying Phosphorylation Activity*

[0832] As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 20, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

[0833] Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degree C until use.

[0834] A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 12 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

[0835] After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound

polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by CRCGCL or a molecule induced by CRCGCL.

*Example 22: Method of Determining Alterations  
in the CRCGCL Gene*

[0836] RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:1. Suggested PCR conditions consist of 35 cycles at 95 degree C for 30 seconds; 60-120 seconds at 52-58 degree C; and 60-120 seconds at 70 degree C, using buffer solutions described in Sidransky, D., et al., Science 252:706 (1991).

[0837] PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons of CRCGCL is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations in CRCGCL is then cloned and sequenced to validate the results of the direct sequencing.

[0838] PCR products of CRCGCL are cloned into T-tailed vectors as described in Holton, T.A. and Graham, M.W., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations in CRCGCL not present in unaffected individuals.

[0839] Genomic rearrangements are also observed as a method of determining alterations in the CRCGCL gene. Genomic clones isolated according to Example 2 are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson, Cg. et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the CRCGCL genomic locus.

[0840] Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise

mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson, Cv. et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region of CRCGCL (hybridized by the probe) are identified as insertions, deletions, and translocations. These CRCGCL alterations are used as a diagnostic marker for an associated disease.

*Example 23: Method of Detecting Abnormal Levels  
of CRCGCL in a Biological Sample*

[0841] CRCGCL polypeptides can be detected in a biological sample, and if an increased or decreased level of CRCGCL is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

[0842] For example, antibody-sandwich ELISAs are used to detect CRCGCL in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies to CRCGCL, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 11. The wells are blocked so that non-specific binding of CRCGCL to the well is reduced.

[0843] The coated wells are then incubated for > 2 hours at RT with a sample containing CRCGCL. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbound CRCGCL.

[0844] Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbound conjugate.

[0845] Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial

dilutions of a control sample, and plot CRCGCL polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the CRCGCL in the sample using the standard curve.

#### *Example 24: Formulating a Polypeptide*

[0846] The CRCGCL composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the CRCGCL polypeptide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

[0847] As a general proposition, the total pharmaceutically effective amount of CRCGCL administered parenterally per dose will be in the range of about 1ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, CRCGCL is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

[0848] Effective dosages of the compositions of the present invention to be administered may be determined through procedures well known to those in the art which address such parameters as biological half-life, bioavailability, and toxicity. Such determination is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0849] Bioexposure of an organism to CRCGCL polypeptide during therapy may also play an important role in determining a therapeutically and/or pharmacologically effective dosing regime. Variations of dosing such as repeated administrations of a relatively low dose of CRCGCL polypeptide for a relatively long period of time may have an effect which is



therapeutically and/or pharmacologically distinguishable from that achieved with repeated administrations of a relatively high dose of CRCGCL for a relatively short period of time.

[0850] Using the equivalent surface area dosage conversion factors supplied by Freireich, E. J., et al. (*Cancer Chemotherapy Reports* 50(4):219-44 (1966)), one of ordinary skill in the art is able to conveniently convert data obtained from the use of CRCGCL in a given experimental system into an accurate estimation of a pharmaceutically effective amount of CRCGCL polypeptide to be administered per dose in another experimental system. Experimental data obtained through the administration of CRCGCL may be converted through the conversion factors supplied by Freireich, et al., to accurate estimates of pharmaceutically effective doses of CRCGCL in rat, monkey, dog, and human. The following conversion table (Table III) is a summary of the data provided by Freireich, et al. Table III gives approximate factors for converting doses expressed in terms of mg/kg from one species to an equivalent surface area dose expressed as mg/kg in another species tabulated.

**Table III.** Equivalent Surface Area Dosage Conversion Factors.

	-TO-				
	Mouse	Rat	Monkey	Dog	Human
-FROM-	(20g)	(150g)	(3.5kg)	(8kg)	(60kg)
Mouse	1	1/2	1/4	1/6	1/12
Rat	2	1	1/2	1/4	1/7
Monkey	4	2	1	3/5	1/3
Dog	6	4	5/3	1	1/2
Human	12	7	3	2	1

[0851] Thus, for example, using the conversion factors provided in Table III, a dose of 50 mg/kg in the mouse converts to an appropriate dose of 12.5 mg/kg in the monkey because  $(50 \text{ mg/kg}) \times (1/4) = 12.5 \text{ mg/kg}$ . As an additional example, doses of 0.02, 0.08, 0.8, 2, and 8 mg/kg in the mouse equate to effect doses of 1.667 micrograms/kg, 6.67 micrograms/kg, 66.7 micrograms/kg, 166.7 micrograms/kg, and 0.667 mg/kg, respectively, in the human.

[0852] Pharmaceutical compositions containing CRCGCL are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by

powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. In one embodiment, "pharmaceutically acceptable carrier" means a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. In a specific embodiment, "pharmaceutically acceptable" means approved by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly humans. Nonlimiting examples of suitable pharmaceutical carriers according to this embodiment are provided in "Remington's Pharmaceutical Sciences" by E. W. Martin, and include sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can be employed as liquid carriers, particularly for injectable solutions. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[0853] The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intrarticular injection and infusion.

[0854] In a preferred embodiment, CRGCL compositions of the invention (including polypeptides, polynucleotides, and antibodies, and agonists and/or antagonists thereof) are administered subcutaneously.

[0855] In another preferred embodiment, CRGCL compositions of the invention (including polypeptides, polynucleotides, and antibodies, and agonists and/or antagonists thereof) are administered intravenously.

[0856] CRGCL is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

[0857] Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and R. Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

[0858] In a preferred embodiment, CRCGCL compositions of the invention are formulated in a biodegradable, polymeric drug delivery system, for example as described in U.S. Patent Nos. 4,938,763; 5,278,201; 5,278,202; 5,324,519; 5,340,849; and 5,487,897 and in International Publication Numbers WO01/35929, WO00/24374, and WO00/06117 which are hereby incorporated by reference in their entirety. In specific preferred embodiments the CRCGCL compositions of the invention are formulated using the ATRIGEL® Biodegradable System of Atrix Laboratories, Inc. (Fort Collins, Colorado).

[0859] Examples of biodegradable polymers which can be used in the formulation of CRCGCL compositions, include but are not limited to, polylactides, polyglycolides, polycaprolactones, polyanhydrides, polyamides, polyurethanes, polyesteramides, polyorthoesters, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, polyphosphazenes, polyhydroxybutyrates, polyhydroxyvalerates, polyalkylene oxalates, polyalkylene succinates, poly(malic acid), poly(amino acids), poly(methyl vinyl ether), poly(maleic anhydride), polyvinylpyrrolidone, polyethylene glycol, polyhydroxycellulose, chitin, chitosan, and copolymers, terpolymers, or combinations or mixtures of the above materials. The preferred polymers are those that have a lower degree of crystallization and are more hydrophobic. These polymers and copolymers are more soluble in the biocompatible solvents than the highly crystalline polymers such as polyglycolide and chitin which also have a high degree of hydrogen-bonding. Preferred materials with the desired solubility parameters are the polylactides, polycaprolactones, and copolymers of these with glycolide in which there are more amorphous regions to enhance solubility. In specific preferred embodiments, the biodegradable polymers which can be used in the formulation of CRCGCL compositions are poly(lactide-co-glycolides). Polymer properties such as molecular weight, hydrophobicity, and lactide/glycolide ratio may be modified to obtain the desired drug CRCGCL release profile (See, e.g., Ravivarapu et al., *Journal of Pharmaceutical Sciences* 89:732-741 (2000), which is hereby incorporated by reference in its entirety).

[0860] It is also preferred that the solvent for the biodegradable polymer be non-toxic, water miscible, and otherwise biocompatible. Examples of such solvents include, but are not limited to, N-methyl-2-pyrrolidone, 2-pyrrolidone, C2 to C6 alkanols, C1 to C15 alcohols, diols, triols, and tetraols such as ethanol, glycerine propylene glycol, butanol; C3 to C15 alkyl ketones such as acetone, diethyl ketone and methyl ethyl ketone; C3 to C15 esters such as methyl acetate, ethyl acetate, ethyl lactate; alkyl ketones such as methyl ethyl ketone, C1 to C15 amides such as dimethylformamide, dimethylacetamide and caprolactam; C3 to C20 ethers such as tetrahydrofuran, or solketal; tweens, triacetin, propylene carbonate, decylmethylsulfoxide, dimethyl sulfoxide, oleic acid, 1-dodecylazacycloheptan-2-one, Other preferred solvents are benzyl alcohol, benzyl benzoate, dipropylene glycol, tributyrin, ethyl oleate, glycerin, glycofural, isopropyl myristate, isopropyl palmitate, oleic acid, polyethylene glycol, propylene carbonate, and triethyl citrate. The most preferred solvents are N-methyl-2-pyrrolidone, 2-pyrrolidone, dimethyl sulfoxide, triacetin, and propylene carbonate because of the solvating ability and their compatibility.

[0861] Additionally, formulations comprising CRCGCL compositions and a biodegradable polymer may also include release-rate modification agents and/or pore-forming agents. Examples of release-rate modification agents include, but are not limited to, fatty acids, triglycerides, other like hydrophobic compounds, organic solvents, plasticizing compounds and hydrophilic compounds. Suitable release rate modification agents include, for example, esters of mono-, di-, and tricarboxylic acids, such as 2-ethoxyethyl acetate, methyl acetate, ethyl acetate, diethyl phthalate, dimethyl phthalate, dibutyl phthalate, dimethyl adipate, dimethyl succinate, dimethyl oxalate, dimethyl citrate, triethyl citrate, acetyl tributyl citrate, acetyl triethyl citrate, glycerol triacetate, di(n-butyl) sebacate, and the like; polyhydroxy alcohols, such as propylene glycol, polyethylene glycol, glycerin, sorbitol, and the like; fatty acids; triesters of glycerol, such as triglycerides, epoxidized soybean oil, and other epoxidized vegetable oils; sterols, such as cholesterol; alcohols, such as C.sub.6 - C.sub.12 alkanols, 2-ethoxyethanol. The release rate modification agent may be used singly or in combination with other such agents. Suitable combinations of release rate modification agents include, but are not limited to, glycerin/propylene glycol, sorbitol/glycerine, ethylene oxide/propylene oxide, butylene glycol/adipic acid, and the like. Preferred release rate modification agents include, but are not limited to, dimethyl citrate, triethyl citrate, ethyl heptanoate, glycerin, and hexanediol. Suitable pore-forming agents that may be used in the

polymer composition include, but are not limited to, sugars such as sucrose and dextrose, salts such as sodium chloride and sodium carbonate, polymers such as hydroxylpropylcellulose, carboxymethylcellulose, polyethylene glycol, and polyvinylpyrrolidone. Solid crystals that will provide a defined pore size, such as salt or sugar, are preferred.

[0862] In specific preferred embodiments the CRCGCL compositions of the invention are formulated using the BEMA™ BioErodible Mucoadhesive System, MCA™ MucoCutaneous Absorption System, SMP™ Solvent MicroParticle System, or BCP™ BioCompatible Polymer System of Atrix Laboratories, Inc. (Fort Collins, Colorado).

[0863] Sustained-release compositions also include liposomally entrapped compositions of the invention (*see generally*, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the CRCGCL are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. USA* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. USA* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal secreted polypeptide therapy.

[0864] In another embodiment sustained release compositions of the invention include crystal formulations known in the art.

[0865] In yet an additional embodiment, the compositions of the invention are delivered by way of a pump (*see Langer, supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)).

[0866] Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

[0867] For parenteral administration, in one embodiment, CRCGCL is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with

other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

[0868] Generally, the formulations are prepared by contacting CRCGCL uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

[0869] The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

[0870] CRCGCL is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

[0871] CRCGCL used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0872] CRCGCL polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized

formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous CRCGCL polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized CRCGCL polypeptide using bacteriostatic Water-for-Injection.

[0873] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, CRCGCL may be employed in conjunction with other therapeutic compounds.

[0874] The compositions of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the compositions of the invention, include but are not limited to, other members of the cytokine receptor family (e.g., IL-7 receptor chain alpha), members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0875] In specific embodiments, the compositions of the invention are administered in combination with members of the type I cytokine receptor family. Type I cytokine receptor family members that may be administered with the compositions of the invention include, but are not limited to, IL-7 receptor chain alpha, IL-2 receptor alpha chain, IL-2 receptor beta chain, IL-4 receptor alpha chain, common alpha chain, common beta chain, and/or IL-13 receptor.

[0876] In a preferred embodiment, the compositions of the invention are administered in combination with IL-7 receptor chain alpha.

[0877] In an additional embodiment, the compositions of the invention are administered in combination with members of the type I cytokine family. Type I cytokine family members that may be administered with the compositions of the invention include, but are not limited to, TSLP, IL-7, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-11, IL-12, IL-13 and/or IL-15.

[0878] In a preferred embodiment, the compositions of the invention are administered in combination with TSLP.

[0879] In one embodiment, the compositions of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the compositions of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-1BB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

[0880] In another specific embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated therewith. In one embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose any Gram positive bacterial infection and/or any disease, disorder, and/or condition associated therewith. In another embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with one or more members of the genus *Enterococcus* and/or the genus *Streptococcus*. In another embodiment, compositions of the



invention are used in any combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with one or more members of the Group B streptococci. In another embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with *Streptococcus pneumoniae*.

[0881] The compositions of the invention may be administered alone or in combination with other therapeutic agents, including but not limited to, chemotherapeutic agents, antibiotics, antivirals, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents and cytokines. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0882] In a further embodiment, the compositions of the invention are administered in combination with CD40 ligand (CD40L), a soluble form of CD40L (e.g., AVREND™), biologically active fragments, variants, or derivatives of CD40L, anti-CD40L antibodies (e.g., agonistic or antagonistic antibodies), and/or anti-CD40 antibodies (e.g., agonistic or antagonistic antibodies).

[0883] In another embodiment, compositions of the invention are administered in combination with an anticoagulant. Anticoagulants that may be administered with the compositions of the invention include, but are not limited to, heparin, warfarin, and aspirin. In a specific embodiment, compositions of the invention are administered in combination with heparin and/or warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin and aspirin. In another specific embodiment, compositions of the invention are administered in combination with heparin. In another specific embodiment, compositions of the invention are administered in combination with heparin and aspirin.

[0884] In another embodiment, compositions of the invention are administered in combination with an agent that suppresses the production of anticardiolipin antibodies. In

specific embodiments, the polynucleotides of the invention are administered in combination with an agent that blocks and/or reduces the ability of anticardiolipin antibodies to bind phospholipid-binding plasma protein beta 2-glycoprotein I (b2GPI).

[0885] In certain embodiments, compositions of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with compositions of the invention to treat, prevent, and/or diagnose AIDS and/or to treat, prevent, and/or diagnose HIV infection.

[0886] In other embodiments, compositions of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the compositions of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, compositions of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat,

prevent, and/or diagnose an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, compositions of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, compositions of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, compositions of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic cytomegalovirus infection. In another specific embodiment, compositions of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat, prevent, and/or diagnose an opportunistic fungal infection. In another specific embodiment, compositions of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, compositions of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, compositions of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat, prevent, and/or diagnose an opportunistic bacterial infection.

[0887] In a further embodiment, the compositions of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the compositions of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

[0888] In a further embodiment, the compositions of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the compositions of the invention include, but are not limited to, amoxicillin, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole,

penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

[0889] Conventional nonspecific immunosuppressive agents, that may be administered in combination with the compositions of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

[0890] In specific embodiments, compositions of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the compositions of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucocorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

[0891] In a preferred embodiment, the compositions of the invention are administered in combination with steroid therapy. Steroids that may be administered in combination with the compositions of the invention, include, but are not limited to, oral corticosteroids, prednisone, and methylprednisolone (e.g., IV methylprednisolone). In a specific embodiment, compositions of the invention are administered in combination with prednisone. In a further specific embodiment, the compositions of the invention are administered in combination with prednisone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the compositions of the invention and prednisone are those described herein, and include, but are not limited to, azathioprine, cyclophosphamide, and cyclophosphamide IV. In another specific embodiment, compositions of the invention are administered in combination with methylprednisolone. In a further specific embodiment, the compositions of the invention are administered in combination with methylprednisolone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the compositions of the invention and methylprednisolone are those described herein, and include, but are not limited to, azathioprine, cyclophosphamide, and cyclophosphamide IV.

[0892] In a preferred embodiment, the compositions of the invention are administered in combination with an antimalarial. Antimalarials that may be administered with the

compositions of the invention include, but are not limited to, hydroxychloroquine, chloroquine, and/or quinacrine.

[0893] In a preferred embodiment, the compositions of the invention are administered in combination with an NSAID.

[0894] In a nonexclusive embodiment, the compositions of the invention are administered in combination with one, two, three, four, five, ten, or more of the following drugs: NRD-101 (Hoechst Marion Roussel), diclofenac (Dimethaid), oxaprozin potassium (Monsanto), mecasemin (Chiron), T-614 (Toyama), pemetrexed disodium (Eli Lilly), atreleuton (Abbott), valdecoxib (Monsanto), eltenac (Byk Gulden), campath, AGM-1470 (Takeda), CDP-571 (Celltech Chiroscience), CM-101 (CarboMed), ML-3000 (Merckle), CB-2431 (KS Biomedix), CBF-BS2 (KS Biomedix), IL-1Ra gene therapy (Valentis), JTE-522 (Japan Tobacco), paclitaxel (Angiotech), DW-166HC (Dong Wha), darbufelone mesylate (Warner-Lambert), soluble TNF receptor 1 (synergen; Amgen), IPR-6001 (Institute for Pharmaceutical Research), trocade (Hoffman-La Roche), EF-5 (Scotia Pharmaceuticals), BIL-284 (Boehringer Ingelheim), BIIF-1149 (Boehringer Ingelheim), LeukoVax (Inflammatics), MK-663 (Merck), ST-1482 (Sigma-Tau), and butixocort propionate (WarnerLambert).

[0895] In a preferred embodiment, the compositions of the invention are administered in combination with one, two, three, four, five or more of the following drugs: methotrexate, sulfasalazine, sodium aurothiomalate, auranofin, cyclosporine, penicillamine, azathioprine, an antimalarial drug (e.g., as described herein), cyclophosphamide, chlorambucil, gold, ENBREL™ (Etanercept), anti-TNF antibody, LJP 394 (La Jolla Pharmaceutical Company, San Diego, California), and prednisolone.

[0896] In a more preferred embodiment, the compositions of the invention are administered in combination with an antimalarial, methotrexate, anti-TNF antibody, ENBREL™ and/or suflasalazine. In one embodiment, the compositions of the invention are administered in combination with methotrexate. In another embodiment, the compositions of the invention are administered in combination with anti-TNF antibody. In another embodiment, the compositions of the invention are administered in combination with methotrexate and anti-TNF antibody. In another embodiment, the compositions of the invention are administered in combination with suflasalazine. In another specific embodiment, the compositions of the invention are administered in combination with

methotrexate, anti-TNF antibody, and suflasalazine. In another embodiment, the compositions of the invention are administered in combination ENBREL™. In another embodiment, the compositions of the invention are administered in combination with ENBREL™ and methotrexate. In another embodiment, the compositions of the invention are administered in combination with ENBREL™, methotrexate and suflasalazine. In another embodiment, the compositions of the invention are administered in combination with ENBREL™, methotrexate and suflasalazine. In other embodiments, one or more antimalarials is combined with one of the above-recited combinations. In a specific embodiment, the compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), ENBREL™, methotrexate and suflasalazine. In another specific embodiment, the compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), sulfasalazine, anti-TNF antibody, and methotrexate.

[0897] In an additional embodiment, compositions of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the compositions of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, compositions of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

[0898] In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the compositions of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

[0899] In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be

administered with the compositions of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepea); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

[0900] In an additional embodiment, the compositions of the invention are administered in combination with cytokines. Cytokines that may be administered with the compositions of the invention include, but are not limited to, GM-CSF, G-CSF, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-alpha, IFN-beta, IFN-gamma, TNF-alpha, and TNF-beta. In another embodiment, compositions of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, and IL-22.

[0901] In an additional embodiment, the compositions of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the compositions of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

[0902] In an additional embodiment, the compositions of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the compositions of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in

Hauser et al., *Growth Factors*, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are incorporated herein by reference herein.

[0903] In an additional embodiment, the compositions of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the compositions of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

[0904] In additional embodiments, the compositions of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy. Such combinatorial therapy may be administered sequentially and/or concomitantly.

#### *Example 25: Method of Treating Decreased Levels of CRCGCL*

[0905] The present invention relates to a method for treating an individual in need of a decreased level of CRCGCL activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of CRCGCL antagonist. Preferred antagonists for use in the present invention are CRCGCL-specific antibodies.

[0906] Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of CRCGCL in an individual can be treated by administering CRCGCL, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of CRCGCL polypeptide



comprising administering to such an individual a pharmaceutical composition comprising an amount of CRCGCL to increase the activity level of CRCGCL in such an individual.

[0907] For example, a patient with decreased levels of CRCGCL polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 24.

#### *Example 26: Method of Treating Increased Levels of CRCGCL*

[0908] The present invention also relates to a method for treating an individual in need of an increased level of CRCGCL activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of CRCGCL or an agonist thereof.

[0909] Antisense technology is used to inhibit production of CRCGCL. This technology is one example of a method of decreasing levels of CRCGCL polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer.

[0910] For example, a patient diagnosed with abnormally increased levels of CRCGCL is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 24.

#### *Example 27: Method of Treatment Using Gene Therapy - Ex Vivo*

[0911] One method of gene therapy transplants fibroblasts, which are capable of expressing CRCGCL polypeptides, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

[0912] At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

[0913] pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

[0914] The cDNA encoding CRCGCL can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector contains properly inserted CRCGCL.

[0915] The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the CRCGCL gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the CRCGCL gene (the packaging cells are now referred to as producer cells).

[0916] Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether CRCGCL protein is produced.

[0917] The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

*Example 28: Gene Therapy Using Endogenous CRCGCL Gene*

[0918] Another method of gene therapy according to the present invention involves operably associating the endogenous CRCGCL sequence with a promoter via homologous recombination as described, for example, in U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

[0919] Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous CRCGCL, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of CRCGCL so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

[0920] The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

[0921] In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be

administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

[0922] Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous CRCGCL sequence. This results in the expression of CRCGCL in the cell. Expression may be detected by immunological staining, or any other method known in the art.

[0923] Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na<sub>2</sub>HPO<sub>4</sub>, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately  $3 \times 10^6$  cells/ml. Electroporation should be performed immediately following resuspension.

[0924] Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the CRCGCL locus, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3' end. Two CRCGCL non-coding sequences are amplified via PCR: one CRCGCL non-coding sequence (CRCGCL fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3' end; the other CRCGCL non-coding sequence (CRCGCL fragment 2) is amplified with a BamHI site at the 5' end and a HindIII site at the 3' end. The CMV promoter and CRCGCL fragments are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; CRCGCL fragment 1 - XbaI; CRCGCL fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

[0925] Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately  $1.5 \times 10^6$  cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V,

respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

[0926] Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

[0927] The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

#### *Example 29: Method of Treatment Using Gene Therapy - In Vivo*

[0928] Another aspect of the present invention is using in vivo gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) CRCGCL sequences into an animal to increase or decrease the expression of the CRCGCL polypeptide. The CRCGCL polynucleotide may be operatively linked to a promoter or any other genetic elements necessary for the expression of the CRCGCL polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata H. et al. (1997) Cardiovasc. Res. 35(3):470-479, Chao J et al. (1997) Pharmacol. Res. 35(6):517-522, Wolff J.A. (1997) Neuromuscul. Disord. 7(5):314-318, Schwartz B. et al. (1996) Gene Ther. 3(5):405-411, Tsurumi Y. et al. (1996) Circulation 94(12):3281-3290 (incorporated herein by reference).

[0929] The CRCGCL polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The CRCGCL

polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

[0930] The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the CRCGCL polynucleotides may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) *Ann. NY Acad. Sci.* 772:126-139 and Abdallah B. et al. (1995) *Biol. Cell* 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

[0931] The CRCGCL polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

[0932] The CRCGCL polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts.

In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

[0933] For the naked CRCGCL polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked CRCGCL polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

[0934] The dose response effects of injected CRCGCL polynucleotide in muscle in vivo is determined as follows. Suitable CRCGCL template DNA for production of mRNA coding for CRCGCL polypeptide is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

[0935] Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The CRCGCL template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

[0936] After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15  $\mu$ m cross-section of the individual quadriceps muscles is histochemically stained for CRCGCL protein expression. A time course for CRCGCL protein expression may be done in a similar fashion except that

quadriceps from different mice are harvested at different times. Persistence of CRCGCL DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using CRCGCL naked DNA.

*Example 30: CRCGCL Transgenic Animals.*

[0937] The CRCGCL polypeptides can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

[0938] Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

[0939] Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated



oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

[0940] The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred.

[0941] Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. The contents of each of the documents recited in this paragraph is herein incorporated by reference in its entirety.

[0942] Any of the CRCGCL polypeptides disclose throughout this application can be used to generate transgenic animals. For example, DNA encoding amino acids M1-K231 of SEQ ID NO:2 can be inserted into a vector containing a promoter, such as the actin promoter, which will ubiquitously express the inserted fragment. Primers that can be used to generate such fragments include a 5' primer containing a BglII restriction site shown in bold:CCGGTTAGATCTGCCATCATGGGGCGGCTGGTTCTGCCGGTTAGATCTGCCATCATGGGGCGGCTGGTTCTGCCGGTTAGATCTGCCATCATGGGGCGGCTGGTTCTG (SEQ ID NO: 32) and a 3' primer, containing a Xba restriction site shown in bold:GGCCGGTCTAGATTATTGGACAGCTTTGGTTTG (SEQ ID NO: 31). This construct

will express the predicted soluble domain of CRCGCL under the control of the actin promoter for ubiquitous expression. The region of CRCGCL included in this construct extends from M1-K231 of SEQ ID NO:2.

[0943] Similarly, the DNA encoding the full length CRCGCL protein can also be inserted into a vector.

[0944] Alternatively, polynucleotides of the invention can be inserted in a vector which controls tissue specific expression through a tissue specific promoter. For example, a construct having a transferrin promoter would express the CRCGCL polypeptide in the liver of transgenic animals. Therefore, DNA encoding amino acids M1-K231 of SEQ ID NO:2 can be amplified using a 5' primer, having a BglII restriction site shown in bold: CCGGTTAGATCTGCCATCATGGGGCGGCTGGTTCTG (SEQ ID NO: 28), and a 3' primer, containing a Xba restriction site shown in bold: GGCCGGTCTAGATTATTTGGACAGCTTTGGTTTG (SEQ ID NO: 31).

[0945] Similarly, the DNA encoding the full length CRCGCL protein can also be inserted into a vector for tissue specific expression.

[0946] Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, in situ hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

[0947] Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of

separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

[0948] Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of CRCGCL polypeptides, studying conditions and/or disorders associated with aberrant CRCGCL expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

### *Example 31: CRCGCL Knock-Out Animals.*

[0949] Endogenous CRCGCL gene expression can also be reduced by inactivating or "knocking out" the CRCGCL gene and/or its promoter using targeted homologous recombination. (E.g., see Smithies et al., Nature 317:230-234 (1985); Thomas & Capecchi, Cell 51:503-512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention in vivo. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (e.g., see Thomas & Capecchi 1987 and Thompson 1989, supra). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site in vivo using appropriate viral vectors that will be apparent to those of skill in the art.

[0950] In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient

in vivo. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered in vitro using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the CRCGCL polypeptides. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

[0951] Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

[0952] When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

[0953] Knock-out animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of CRCGCL polypeptides, studying conditions and/or disorders associated with aberrant CRCGCL expression, and in screening for compounds effective in ameliorating such conditions and/or disorders. For example, a knock-out mouse can be made using the sequences disclosed as AA008694 and W98372, herein incorporated by reference in their entirety.

*Example 32: Assays Detecting Stimulation or Inhibition of  
B cell Proliferation and Differentiation*

[0954] Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

[0955] One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors:

[0956] **In Vitro Assay-** Purified CRGCL protein, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of CRGCL protein on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

[0957] Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added  $10^5$  B-cells suspended in culture medium (RPMI 1640 containing 10% FBS,  $5 \times 10^{-5}$  M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and  $10^{-5}$  dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with  $^3\text{H}$ -thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

[0958] **In Vivo Assay-** BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of CRCGCL protein, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal and CRCGCL protein-treated spleens identify the results of the activity of CRCGCL protein on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

[0959] Flow cytometric analyses of the spleens from CRCGCL protein-treated mice is used to indicate whether CRCGCL protein specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

[0960] Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and CRCGCL protein-treated mice.

[0961] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

### *Example 33: T Cell Proliferation Assay*

[0962] A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of  $^3\text{H}$ -thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100  $\mu\text{l}$ /well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1)

overnight at 4°C (1 µg/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells ( $5 \times 10^4$ /well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of CRCGCL protein (total volume 200 µl). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37°C, plates are spun for 2 min. at 1000 rpm and 100 µl of supernatant is removed and stored -20°C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 µl of medium containing 0.5 µCi of  $^3\text{H}$ -thymidine and cultured at 37°C for 18-24 hr. Wells are harvested and incorporation of  $^3\text{H}$ -thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of CRCGCL proteins.

[0963] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

*Example 34: Effect of CRCGCL on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells*

[0964] Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF- $\alpha$ , causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FCyRII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

[0965] FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of CRCGCL or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4°C. After an

additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

[0966]      Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells ( $10^6/\text{ml}$ ) are treated with increasing concentrations of CRCGCL for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

[0967]      Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

[0968]      FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of CRCGCL or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4°C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

[0969]      Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. CRCGCL, agonists, or antagonists of CRCGCL can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from



single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

[0970]      Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of  $2 \times 10^6$ /ml in PBS containing PI at a final concentration of 5 µg/ml, and then incubated at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

[0971]      Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of  $5 \times 10^5$  cells/ml with increasing concentrations of CRCGCL and under the same conditions, but in the absence of CRCGCL. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of CRCGCL. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g, R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

[0972]      Oxidative burst. Purified monocytes are plated in 96-w plate at  $2 \times 10^5$  cell/well. Increasing concentrations of CRCGCL are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0,

5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 µl 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of H<sub>2</sub>O<sub>2</sub> produced by the macrophages, a standard curve of a H<sub>2</sub>O<sub>2</sub> solution of known molarity is performed for each experiment.

[0973] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

### *Example 35: CRCGCL Biological Effects*

#### **Astrocyte and Neuronal Assays.**

[0974] Recombinant CRCGCL, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate CRCGCL's activity on these cells.

[0975] Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke, P. et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of CRCGCL to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

#### **Fibroblast and endothelial cell assays.**

[0976] Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE<sub>2</sub> assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or CRGCL with or without IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for PGE<sub>2</sub> by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or CRGCL with or without IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

[0977] Human lung fibroblasts are cultured with FGF-2 or CRGCL for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with CRGCL.

#### **Parkinson Models.**

[0978] The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP<sup>+</sup>) and released. Subsequently, MPP<sup>+</sup> is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP<sup>+</sup> is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotinamide adenine

disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

[0979] It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

[0980] Based on the data with FGF-2, CRCGCL can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of CRCGCL is first examined *in vitro* in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm<sup>2</sup> on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

[0981] Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if CRCGCL acts to prolong the survival of dopaminergic neurons, it would suggest that CRCGCL may be involved in Parkinson's Disease.

[0982] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

*Example 36: The Effect of CRCGCL on the Growth of  
Vascular Endothelial Cells*

[0983] On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at  $2-5 \times 10^4$  cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. CRCGCL protein of SEQ ID NO. 2, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

[0984] An increase in the number of HUVEC cells indicates that CRCGCL may proliferate vascular endothelial cells.

[0985] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

*Example 37: Stimulatory Effect of CRCGCL on the Proliferation  
of Vascular Endothelial Cells*

[0986] For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1 mL serum-supplemented medium and are allowed to attach overnight. After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, VEGF<sub>165</sub> or CRCGCL in 0.5% FBS) with or without Heparin (8 U/ml) are added to wells for 48 hours. 20 mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490 nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is subtracted, and seven wells are performed in parallel for each condition. See, Leak *et al. In Vitro Cell. Dev. Biol.* 30A:512-518 (1994).

[0987] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

*Example 38: Inhibition of PDGF-induced Vascular Smooth Muscle Cell Proliferation Stimulatory Effect*

[0988] HAoSMC proliferation can be measured, for example, by BrdUrd incorporation. Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with 10% calf serum and 6 mg/ml BrdUrd. After 24 h, immunocytochemistry is performed by using BrdUrd Staining Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdUrd antibody at 4 °C for 2 h after being exposed to denaturing solution and then incubated with the streptavidin-peroxidase and diaminobenzidine. After counterstaining with hematoxylin, the cells are mounted for microscopic examination, and the BrdUrd-positive cells are counted. The BrdUrd index is calculated as a percent of the BrdUrd-positive cells to the total cell number. In addition, the simultaneous detection of the BrdUrd staining (nucleus) and the FITC uptake (cytoplasm) is performed for individual cells by the concomitant use of bright field illumination and dark field-UV fluorescent illumination. See, Hayashida et al., J. Biol. Chem. 6:271(36):21985-21992 (1996).

[0989] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

*Example 39: Stimulation of Endothelial Migration*

[0990] This example will be used to explore the possibility that CRCGCL may stimulate lymphatic endothelial cell migration.

[0991] Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 um (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours at room temperature and dried under sterile air. Test substances are diluted to appropriate concentrations

in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25 ul of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber,  $2.5 \times 10^5$  cells suspended in 50 ul M199 containing 1% FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO<sub>2</sub> to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

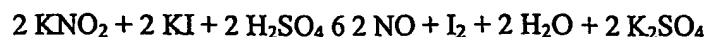
[0992] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

#### *Example 40: Stimulation of Nitric Oxide Production by Endothelial Cells*

[0993] Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, CRCGCL activity can be assayed by determining nitric oxide production by endothelial cells in response to CRCGCL.

[0994] Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and CRCGCL. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of CRCGCL on nitric oxide release is examined on HUVEC.

[0995] Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:



[0996] The standard calibration curve is obtained by adding graded concentrations of KNO<sub>2</sub> (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and H<sub>2</sub>SO<sub>4</sub>. The specificity of the Iso-NO electrode to NO is previously determined by measurement

of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C. The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions. S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per  $1 \times 10^6$  endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak *et al. Biochem. and Biophys. Res. Comm.* 217:96-105 (1995).

[0997] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

#### *Example 41: Effect of CRCGCL on Cord Formation in Angiogenesis*

[0998] Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured *in vitro*.

[0999] CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the *in vitro* angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200 µl/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300 µg Cell Applications' Cord Formation Medium containing control buffer or CRCGCL (0.1 to 100 ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in triplicate.

[1000] Commercial (R&D) VEGF (50 ng/ml) is used as a positive control.  $\beta$ -estradiol (1 ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.



[1001] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

#### *Example 42: Angiogenic Effect on Chick Chorioallantoic Membrane*

[1002] Chick chorioallantoic membrane (CAM) is a well-established system to examine angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The ability of CRCGCL to stimulate angiogenesis in CAM can be examined.

[1003] Fertilized eggs of the White Leghorn chick (*Gallus gallus*) and the Japanese quail (*Coturnix coturnix*) are incubated at 37.8°C and 80% humidity. Differentiated CAM of 16-day-old chick and 13-day-old quail embryos is studied with the following methods.

[1004] On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5 mm in diameter. Sterile and salt-free growth factors are dissolved in distilled water and about 3.3 mg/ 5 ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer. They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

[1005] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

#### *Example 43: Angiogenesis Assay Using a Matrigel Implant in Mouse*

[1006] *In vivo* angiogenesis assay of CRCGCL measures the ability of an existing capillary network to form new vessels in an implanted capsule of murine extracellular matrix material (Matrigel). The protein is mixed with the liquid Matrigel at 4 degree C and the mixture is then injected subcutaneously in mice where it solidifies. After 7 days, the solid "plug" of

Matrigel is removed and examined for the presence of new blood vessels. Matrigel is purchased from Becton Dickinson Labware/Collaborative Biomedical Products.

[1007] When thawed at 4 degree C the Matrigel material is a liquid. The Matrigel is mixed with CRCGCL at 150 ng/ml at 4 degree C and drawn into cold 3 ml syringes. Female C57Bl/6 mice approximately 8 weeks old are injected with the mixture of Matrigel and experimental protein at 2 sites at the midventral aspect of the abdomen (0.5 ml/site). After 7 days, the mice are sacrificed by cervical dislocation, the Matrigel plugs are removed and cleaned (i.e., all clinging membranes and fibrous tissue is removed). Replicate whole plugs are fixed in neutral buffered 10% formaldehyde, embedded in paraffin and used to produce sections for histological examination after staining with Masson's Trichrome. Cross sections from 3 different regions of each plug are processed. Selected sections are stained for the presence of vWF. The positive control for this assay is bovine basic FGF (150 ng/ml). Matrigel alone is used to determine basal levels of angiogenesis.

[1008] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

#### *Example 44: Rescue of Ischemia in Rabbit Lower Limb Model*

[1009] To study the in vivo effects of CRCGCL on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously (Takeshita, S. *et al.*, *Am J. Pathol* 147:1649-1660 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshita, S. *et al.* *Am J. Pathol* 147:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked CRCGCL expression plasmid by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen, R. *et al.* *Hum Gene Ther.* 4:749-758 (1993); Leclerc, G. *et al.* *J. Clin. Invest.* 90: 936-944 (1992)). When CRCGCL is used in the treatment, a single bolus of 500 mg CRCGCL protein or control is

delivered into the internal iliac artery of the ischemic limb over a period of 1 min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number in the rabbit thigh; (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

[1010] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

#### *Example 45: Effect of CRCGCL on Vasodilation*

[1011] Since dilation of vascular endothelium is important in reducing blood pressure, the ability of CRCGCL to affect the blood pressure in spontaneously hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900 mg/kg) of the CRCGCL are administered to 13-14 week old spontaneously hypertensive rats (SHR). Data are expressed as the mean  $\pm$  SEM. Statistical analysis are performed with a paired t-test and statistical significance is defined as  $p < 0.05$  vs. the response to buffer alone.

[1012] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

#### *Example 46: Rat Ischemic Skin Flap Model*

[1013] The evaluation parameters include skin blood flow, skin temperature, and factor VIII immunohistochemistry or endothelial alkaline phosphatase reaction. CRCGCL expression, during the skin ischemia, is studied using in situ hybridization.

[1014] The study in this model is divided into three parts as follows:

Ischemic skin

Ischemic skin wounds and

Normal wounds.

[1015] The experimental protocol includes:

a) raising a 3x4 cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal);

b) an excisional wounding (4-6 mm in diameter) in the ischemic skin (skin-flap);

c) topical treatment with CRCGCL of the excisional wounds (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100 mg; and

d) harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for histological, immunohistochemical, and in situ studies.

[1016] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

#### *Example 47: Peripheral Arterial Disease Model*

[1017] Angiogenic therapy using CRCGCL is a novel therapeutic strategy to obtain restoration of blood flow around the ischemia in case of peripheral arterial diseases. The experimental protocol includes:

a) one side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control;

b) CRCGCL protein, in a dosage range of 20 mg to 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3 weeks;

c) the ischemic muscle tissue is collected after ligation of the femoral artery at 1, 2, and 3 weeks for the analysis of CRCGCL expression and histology; and

d) biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

[1018] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

#### *Example 48: Ischemic Myocardial Disease Model*

[1019] CRCGCL is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of CRCGCL expression is investigated in situ. The experimental protocol includes:

The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

CRCGCL protein, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

[1020] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

#### *Example 49: Rat Corneal Wound Healing Model*

[1021] This animal model shows the effect of CRCGCL on neovascularization. The experimental protocol includes:

- a) making a 1-1.5 mm long incision from the center of cornea into the stromal layer;
- b) inserting a spatula below the lip of the incision facing the outer corner of the eye;
- c) making a pocket (its base is 1-1.5 mm from the edge of the eye); and
- d) positioning a pellet, containing 50ng- 5ug of CRCGCL, within the pocket.
- e) CRCGCL treatment can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

[1022] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

#### *Example 50: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models*

*Diabetic db+/db+ Mouse Model.*

[1023] To demonstrate that CRCGCL accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

[1024] The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al.* *Proc. Natl. Acad. Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am. J. of Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.* 83(2):221-232 (1984); Robertson *et al.*, *Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*, *Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

[1025] The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of Pathol.* 136:1235-1246 (1990)).

[1026] Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

[1027] Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of

wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

[1028] Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

[1029] CRCGCL is administered using at a range different doses of CRCGCL, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

[1030] Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

[1031] Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) CRCGCL.

[1032] Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

[1033] Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with CRCGCL. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

[1034] Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

[1035] Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer served as a positive tissue control and human brain tissue is used as a negative tissue control. Each specimen included a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

[1036] Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

#### ***Steroid Impaired Rat Model***

[1037] The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, S.M. Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl, S.M.*et al.*, *J. Immunol.* 115: 476-481 (1975); Werb, Z. *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability ( Ebert, R.H., *et al.*, *An. Intern. Med.* 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck, L.S. *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes, B.F. *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating



monocytes (Haynes, B.F., et al., *J. Clin. Invest.* 61: 703-797 (1978); Wahl, S. M., "Glucocorticoids and wound healing", *In: Antiinflammatory Steroid Action: Basic and Clinical Aspects*, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck, L.S. et al., *Growth Factors.* 5: 295-304 (1991); Haynes, B.F., et al., *J. Clin. Invest.* 61: 703-797 (1978); Wahl, S. M., "Glucocorticoids and wound healing", *In: Antiinflammatory Steroid Action: Basic and Clinical Aspects*, Academic Press, New York, pp. 280-302 (1989); Pierce, G.F. et al., *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).

[1038] To demonstrate that CRCGCL can accelerate the healing process, the effects of multiple topical applications of CRCGCL on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

[1039] Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

[1040] The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

[1041] Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated

Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

[1042] CRCGCL is administered using at a range different doses of CRCGCL, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

[1043] Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

[1044] Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) CRCGCL treated groups.

[1045] Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

[1046] Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with CRCGCL. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

[1047] Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

[1048] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

### *Example 51: Lymphadema Animal Model*

[1049] The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of CRCGCL in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

[1050] Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

[1051] Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated or suture ligated.

[1052] Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

[1053] Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

[1054] To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

[1055] **Circumference Measurements:** Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

[1056] **Volumetric Measurements:** On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

[1057] **Blood-plasma protein measurements:** Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and  $Ca^{2+}$  comparison.

[1058] **Limb Weight Comparison:** After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

[1059] **Histological Preparations:** The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

[1060] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

*Example 52: Suppression of TNF alpha-induced adhesion molecule expression by CRCGCL*

[1061] The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

[1062] Tumor necrosis factor alpha (TNF- $\alpha$ ), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

[1063] The potential of CRCGCL to mediate a suppression of TNF- $\alpha$  induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF- $\alpha$  treated ECs when co-stimulated with a member of the FGF family of proteins.

[1064] To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO<sub>2</sub>. HUVECs are seeded in 96-well plates at concentrations of  $1 \times 10^4$  cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

[1065] Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90  $\mu$ l of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the

plate in triplicate (in 10  $\mu$ l volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ ) is added to each well. Plates are held at 4°C for 30 min.

[1066] Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10  $\mu$ l of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

[1067] Then add 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100  $\mu$ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 ( $10^0$ ) >  $10^{-0.5}$  >  $10^{-1}$  >  $10^{-1.5}$ . 5  $\mu$ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100  $\mu$ l of pNPP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50  $\mu$ l of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

[1068] The studies described in this example tested activity in CRCGCL protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of CRCGCL polynucleotides (e.g., gene therapy), agonists, and/or antagonists of CRCGCL.

*Example 53: Induction of T Cell Growth from Bone Marrow  
By CRCGCL agonists and IL-7*

[1069] CRCGCL polypeptides of the invention and/or agonists thereof are tested for the induction of outgrowth of T cells from human bone marrow.

[1070] Human bone marrow-derived mononuclear cells (BM MNC) are isolated by centrifugation of whole bone marrow over Ficoll. BM MNC are cultured in McCoy's media supplemented with 10% fetal bovine serum, and amino acid and vitamin supplements, at a concentration ranging between  $4.5 \times 10^5$  to  $10^6$  cells/ml in a total volume of 6 or 7 ml per flask (T25). CRCGCL polypeptides of the invention and/or agonists thereof and other cytokines, i.e., IL-7, SLF (i.e., steel factor or stem cell factor, or mast cell growth factor), or flt3L, either alone or in combination, are added to the cultures at day 0. After 14 days and weekly thereafter, half the culture is removed for counting. Fresh media and cytokines are added to the cultures to return the total volume to 6 or 7 ml.

[1071] Harvested cells are also analyzed via flow cytometry fourteen days after culture and weekly thereafter, using antibodies specific for cell surface antigens. The antibodies used may be specific for T cell antigens (i.e., the alpha/beta T cell receptor, gamma/delta T cell receptor, and CD3), B cell antigens (i.e., CD 19 and surface IgM), Natural Killer cell antigens (i.e., CD56), monocyte antigens (i.e., CD14), and granulocyte antigens (i.e., CD15).

[1072] Addition of CRCGCL polypeptides of the invention, and/or agonists thereof and IL-7 to BM MNC cultures induce cellular growth.

#### *Example 54: 293T Reconstitution System*

[1073] This assay was used to assess whether the CRCGCL polypeptides can mediate tyrosine phosphorylation of the Jak-STAT pathway, as has been shown for the IL-2R gamma chain (common gamma).

[1074] 293T cells were transfected by the  $\text{Ca}_2\text{PO}_4$  method, using 2  $\mu\text{g}$  of each construct, never to exceed 10  $\mu\text{g}$  per plate. The medium was changed after 16 hours, and cells were cultured in complete DMEM for an additional 24 hours. Cells were then stimulated with the cytokine of interest: IL-7, IL-2, or TSLP, washed in PBS, and lysed in Brij lysis buffer (10mM Tris, pH 7.5, 0.875% Brij, 0.125% NP40, 2.0mM EDTA, 150mM NaCl). 300-500  $\mu\text{g}$  of total protein was immunoprecipitated with the antibody of interest (STAT5 or Jak 2, Santa Cruz Biotechnology, Inc. Santa Cruz, CA), separated on 8% SDS-PAGE gels and transferred to Immobilon-P membranes. The membranes were blotted with anti-phosphotyrosine antibody 4G10 (UBI), and detected using chemiluminescence.

**[1075]** Increased phosphorylation of STAT5 and Tyk2 was detected using this assay when CRCGCL was cotransfected with IL-7R alpha chain, Jak2, and STAT5 (data not shown). No effect was seen with vector alone or when CRCGCL was cotransfected with Jak3, common gamma chain, or with the IL-2 beta chain.

**[1076]** It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

**[1077]** The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Moreover, the disclosure and sequence listing from U.S. application Serial Nos. 60/078,563, filed March 19, 1998, 60/086,505, filed May 22, 1998, 09/263,626, filed March 5, 1999, 09/376,430, filed August 18, 1999, and 60/269,876, filed February 21, 2001; and International Application Nos. PCT/US99/05069, filed March 5, 1999, and PCT/US00/22493 filed August 17, 2000, are herein incorporated by reference in their entireties.



### INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

**(PCT Rule 13*bis*)**

**A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 3, Paragraph 10.**

### B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☒

**Name of depositary institution:** American Type Culture Collection

**Address of depositary institution (including postal code and country)**

**10801 University Boulevard**

Manassas, Virginia 20110-2209

United States of America

Date of deposit

**February 25, 1998**

Accession Number

**209641**

**C. ADDITIONAL INDICATIONS** *(leave blank if not applicable)*

**This information is continued on an additional sheet** ☐

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)

## Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

**E. SEPARATE FURNISHING OF INDICATIONS** *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

**For receiving Office use only**

**For International Bureau use only**

☒ This sheet was received with the international application

☐ This sheet was received by the International Bureau on:

Authorized officer, Immigration Processing Division

**Authorized officer**

**ATCC Deposit No. 209641**

**CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**ATCC Deposit No.: 209641**

## **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

## **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

## **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by an applicant in the individual case.

## **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

**A.** The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 3, Paragraph 10.

**B. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet ☐

Name of depositary institution: American Type Culture Collection

Address of depositary institution (including postal code and country)

10801 University Boulevard  
Manassas, Virginia 20110-2209  
United States of America

Date of deposit

March 23, 1998

Accession Number

209691

**C. ADDITIONAL INDICATIONS** (leave blank if not applicable)

This information is continued on an additional sheet ☐

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

For receiving Office use only				For International Bureau use only	
<input checked="" type="checkbox"/> This sheet was received with the international application			<input type="checkbox"/> This sheet was received by the International Bureau on:		
Authorized officer <u>Larry Hammond, Paralegal</u> <u>International Application Processing Division</u>			Authorized officer		

**ATCC Deposit No. 209691**

**CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**ATCC Deposit No.: 209691**

## **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

## **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

## **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by an applicant in the individual case.

## **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

*What Is Claimed Is:*

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 90% or more identical to a sequence selected from the group consisting of:
  - (a) a polynucleotide fragment of SEQ ID NO:1 or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No: 209641 or 209691;
  - (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209641 or 209691;
  - (c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209641 or 209691;
  - (d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209641 or 209691;
  - (e) a polynucleotide encoding a polypeptide of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209641 or 209691 having biological activity;
  - (f) a polynucleotide which is a variant of SEQ ID NO:1;
  - (g) a polynucleotide which is an allelic variant of SEQ ID NO:1;
  - (h) a polynucleotide which encodes a species homologue of the SEQ ID NO:2;and
  - (i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.
2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a mature form or a secreted protein.
3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:2 or the coding sequence included in ATCC Deposit No: 209641 or 209691.

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:1 or the cDNA sequence included in ATCC Deposit No: 209641 or 209691.
5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.
6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.
7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.
8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.
9. A recombinant host cell produced by the method of claim 9.
10. The recombinant host cell of claim 9 comprising vector sequences.
11. An isolated polypeptide comprising an amino acid sequence at least 90% or more identical to a sequence selected from the group consisting of:
  - (a) a polypeptide fragment of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209641 or 209691;
  - (b) a polypeptide fragment of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209641 or 209691 having biological activity;
  - (c) a polypeptide domain of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209641 or 209691;
  - (d) a polypeptide epitope of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209641 or 209691;
  - (e) a mature form of a secreted protein;
  - (f) a full length secreted protein;
  - (g) a variant of SEQ ID NO:2;



- (h) an allelic variant of SEQ ID NO:2; and
  - (i) a species homologue of the SEQ ID NO:2.
- 12. The isolated polypeptide of claim 11, wherein the mature form or the full length secreted protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.
- 13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.
- 14. A recombinant host cell that expresses the isolated polypeptide of claim 11.
- 15. A method of making an isolated polypeptide comprising:
  - (a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and
  - (b) recovering said polypeptide.
- 16. The polypeptide produced by claim 15.
- 17. A method for preventing, treating, or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11.
- 18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject related to expression or activity of a secreted protein comprising:
  - (a) determining the presence or absence of a mutation in the polynucleotide of claim 1;
  - (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.
- 19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject related to expression or activity of a secreted protein comprising:

(a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample;

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying binding partner to the polypeptide of claim 11 comprising:

(a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

21. The gene corresponding to the cDNA sequence of SEQ ID NO:2.

22. A method of identifying an activity in a biological assay, wherein the method comprises:

(a) expressing SEQ ID NO:1 in a cell;

(b) isolating the supernatant;

(c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 22

24. A multimer comprising the polypeptide of claim 11.

## SEQUENCE LISTING

&lt;110&gt; Human Genome Sciences, Inc.

&lt;120&gt; Cytokine Receptor Common Gamma Chain Like

&lt;130&gt; PF466PCT3

&lt;150&gt; 60/269,876

&lt;151&gt; 2001-02-21

&lt;160&gt; 32

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 1573

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (13)..(1125)

&lt;400&gt; 1

cggcacgagg gc atg ggg cgg ctg gtt ctg ctg tgg gga gct gcc gtc ttt 51  
 Met Gly Arg Leu Val Leu Leu Trp Gly Ala Ala Val Phe  
                   1                                  5                                  10

ctg ctg gga ggc tgg atg gct ttg ggg caa gga gga gca gca gaa gga 99  
 Leu Leu Gly Gly Trp Met Ala Leu Gly Gln Gly Gly Ala Ala Glu Gly  
           15                                  20                                  25

gta cag att cag atc atc tac ttc aat tta gaa acc gtg cag gtg aca 147  
 Val Gln Ile Gln Ile Ile Tyr Phe Asn Leu Glu Thr Val Gln Val Thr  
           30                                  35                                  40                                  45

tgg aat gcc agc aaa tac tcc agg acc aac ctg act ttc cac tac aga 195  
 Trp Asn Ala Ser Lys Tyr Ser Arg Thr Asn Leu Thr Phe His Tyr Arg  
                                   50                                  55                                  60

ttc aac ggt gat gag gcc tat gac cag tgc acc aac tac ctt ctc cag 243  
 Phe Asn Gly Asp Glu Ala Tyr Asp Gln Cys Thr Asn Tyr Leu Leu Gln  
                                   65                                  70                                  75

gaa ggt cac act tcg ggg tgc ctc cta gac gca gag cag cga gac gac 291  
 Glu Gly His Thr Ser Gly Cys Leu Leu Asp Ala Glu Gln Arg Asp Asp  
                                   80                                  85                                  90

att ctc tat ttc tcc atc agg aat ggg acg cac ccc gtt ttc acc gca 339  
 Ile Leu Tyr Phe Ser Ile Arg Asn Gly Thr His Pro Val Phe Thr Ala  
                                   95                                  100                                  105

agt cgc tgg atg gtt tat tac ctg aaa ccc agt tcc ccg aag cac gtg 387  
 Ser Arg Trp Met Val Tyr Tyr Leu Lys Pro Ser Ser Pro Lys His Val  
 110                                  115                                  120                                  125

aga ttt tcg tgg cat cag gat gca gtg acg gtg acg tgt tct gac ctg 435  
 Arg Phe Ser Trp His Gln Asp Ala Val Thr Val Thr Cys Ser Asp Leu

130	135	140	
tcc tac ggg gat ctc ctc tat gag gtt cag tac cgg agc ccc ttc gac Ser Tyr Gly Asp Leu Leu Tyr Glu Val Gln Tyr Arg Ser Pro Phe Asp 145 150 155			483
acc gag tgg cag tcc aaa cag gaa aat acc tgc aac gtc acc ata gaa Thr Glu Trp Gln Ser Lys Gln Glu Asn Thr Cys Asn Val Thr Ile Glu 160 165 170			531
ggc ttg gat gcc gag aag tgt tac tct ttc tgg gtc agg gtg aag gct Gly Leu Asp Ala Glu Lys Cys Tyr Ser Phe Trp Val Arg Val Lys Ala 175 180 185			579
atg gag gat gta tat ggg cca gac aca tac cca agc gac tgg tca gag Met Glu Asp Val Tyr Gly Pro Asp Thr Tyr Pro Ser Asp Trp Ser Glu 190 195 200 205			627
gtg aca tgc tgg cag aga ggc gag att cgg gat gcc tgt gca gag aca Val Thr Cys Trp Gln Arg Gly Glu Ile Arg Asp Ala Cys Ala Glu Thr 210 215 220			675
cca acg cct ccc aaa cca aag ctg tcc aaa ttt att tta att tcc agc Pro Thr Pro Pro Lys Pro Lys Leu Ser Lys Phe Ile Leu Ile Ser Ser 225 230 235			723
ctg gcc atc ctt ctg atg gtg tct ctc ctc ctt ctg tct tta tgg aaa Leu Ala Ile Leu Leu Met Val Ser Leu Leu Leu Leu Ser Leu Trp Lys 240 245 250			771
tta tgg aga gtg aag aag ttt ctc att ccc agc gtg cca gac ccg aaa Leu Trp Arg Val Lys Lys Phe Leu Ile Pro Ser Val Pro Asp Pro Lys 255 260 265			819
tcc atc ttc ccc ggg ctc ttt gag ata cac caa ggg aac ttc cag gag Ser Ile Phe Pro Gly Leu Phe Glu Ile His Gln Gly Asn Phe Gln Glu 270 275 280 285			867
tgg atc aca gac acc cag aac gtg gcc cac ctc cac aag atg gca ggt Trp Ile Thr Asp Thr Gln Asn Val Ala His Leu His Lys Met Ala Gly 290 295 300			915
gca gag caa gaa agt ggc ccc gag gag ccc ctg gta gtc cag ttg gcc Ala Glu Gln Glu Ser Gly Pro Glu Glu Pro Leu Val Val Gln Leu Ala 305 310 315			963
aag act gaa gcc gag tct ccc agg atg ctg gac cca cag acc gag gag Lys Thr Glu Ala Glu Ser Pro Arg Met Leu Asp Pro Gln Thr Glu Glu 320 325 330			1011
aaa gag gcc tct ggg gga tcc ctc cag ctt ccc cac cag ccc ctc caa Lys Glu Ala Ser Gly Gly Ser Leu Gln Leu Pro His Gln Pro Leu Gln 335 340 345			1059
ggc ggt gat gtg gtc aca atc ggg ggc ttc acc ttt gtg atg aat gac Gly Gly Asp Val Val Thr Ile Gly Gly Phe Thr Phe Val Met Asn Asp 350 355 360 365			1107
cgc tcc tac gtg gcg ttg tgatggacac accactgtca aagtcaacgt			1155

Arg Ser Tyr Val Ala Leu  
370

caggatccac gttgacattt aaagacagag gggactgtcc cggggactcc acaccacccat 1215  
ggatgggaag tctccacgcc aatgatggta ggactaggag actctgaaga cccagcctca 1275  
ccgcctaata cgccactgc cctgca.aact ttccccca ca tgagtctctg tgttcaaagg 1335  
cttgatggca gatgggagcc aattgctcca ggagatttac tcccagttcc ttttcgtgcc 1395  
tgaacgttgt cacataaacc ccaaggcagc acgtccaaaa tgctgtaaaa ccatcttccc 1455  
actctgtgag tccccagttc cgtccatgta cctgttccat agcattggat tctcggagga 1515  
ttttttgtct gttttgagac tccaaaccac ctctaccctt aaaaaaaaaa aaaaaaaaaa 1573

<210> 2  
<211> 371  
<212> PRT  
<213> Homo sapiens

<400> 2  
Met Gly Arg Leu Val Leu Leu Trp Gly Ala Ala Val Phe Leu Leu Gly  
1 5 10 15

Gly Trp Met Ala Leu Gly Gln Gly Gly Ala Ala Glu Gly Val Gln Ile  
20 25 30

Gln Ile Ile Tyr Phe Asn Leu Glu Thr Val Gln Val Thr Trp Asn Ala  
35 40 45

Ser Lys Tyr Ser Arg Thr Asn Leu Thr Phe His Tyr Arg Phe Asn Gly  
50 55 60

Asp Glu Ala Tyr Asp Gln Cys Thr Asn Tyr Leu Leu Gln Glu Gly His  
65 70 75 80

Thr Ser Gly Cys Leu Leu Asp Ala Glu Gln Arg Asp Asp Ile Leu Tyr  
85 90 95

Phe Ser Ile Arg Asn Gly Thr His Pro Val Phe Thr Ala Ser Arg Trp  
100 105 110

Met Val Tyr Tyr Leu Lys Pro Ser Ser Pro Lys His Val Arg Phe Ser  
115 120 125

Trp His Gln Asp Ala Val Thr Val Thr Cys Ser Asp Leu Ser Tyr Gly  
130 135 140

Asp Leu Leu Tyr Glu Val Gln Tyr Arg Ser Pro Phe Asp Thr Glu Trp  
145 150 155 160

Gln Ser Lys Gln Glu Asn Thr Cys Asn Val Thr Ile Glu Gly Leu Asp  
165 170 175

Ala Glu Lys Cys Tyr Ser Phe Trp Val Arg Val Lys Ala Met Glu Asp  
180 185 190

Val Tyr Gly Pro Asp Thr Tyr Pro Ser Asp Trp Ser Glu Val Thr Cys  
 195 200 205  
 Trp Gln Arg Gly Glu Ile Arg Asp Ala Cys Ala Glu Thr Pro Thr Pro  
 210 215 220  
 Pro Lys Pro Lys Leu Ser Lys Phe Ile Leu Ile Ser Ser Leu Ala Ile  
 225 230 235 240  
 Leu Leu Met Val Ser Leu Leu Leu Leu Ser Leu Trp Lys Leu Trp Arg  
 245 250 255  
 Val Lys Lys Phe Leu Ile Pro Ser Val Pro Asp Pro Lys Ser Ile Phe  
 260 265 270  
 Pro Gly Leu Phe Glu Ile His Gln Gly Asn Phe Gln Glu Trp Ile Thr  
 275 280 285  
 Asp Thr Gln Asn Val Ala His Leu His Lys Met Ala Gly Ala Glu Gln  
 290 295 300  
 Glu Ser Gly Pro Glu Glu Pro Leu Val Val Gln Leu Ala Lys Thr Glu  
 305 310 315 320  
 Ala Glu Ser Pro Arg Met Leu Asp Pro Gln Thr Glu Glu Lys Glu Ala  
 325 330 335  
 Ser Gly Gly Ser Leu Gln Leu Pro His Gln Pro Leu Gln Gly Gly Asp  
 340 345 350  
 Val Val Thr Ile Gly Gly Phe Thr Phe Val Met Asn Asp Arg Ser Tyr  
 355 360 365  
 Val Ala Leu  
 370

&lt;210&gt; 3

&lt;211&gt; 379

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 3

Met Leu Lys Pro Pro Leu Pro Leu Arg Ser Leu Leu Phe Leu Gln Leu  
 1 5 10 15  
 Pro Leu Leu Gly Val Gly Leu Asn Pro Lys Phe Leu Thr Pro Ser Gly  
 20 25 30  
 Asn Glu Asp Ile Gly Gly Lys Pro Gly Thr Gly Gly Asp Phe Phe Leu  
 35 40 45  
 Thr Ser Thr Pro Ala Gly Thr Leu Asp Val Ser Thr Leu Pro Leu Pro  
 50 55 60  
 Lys Val Gln Cys Phe Val Phe Asn Val Glu Tyr Met Asn Cys Thr Trp  
 65 70 75 80

Asn Ser Ser Ser Glu Pro Gln Pro Asn Asn Leu Thr Leu His Tyr Gly  
                     85                                    90                                    95  
 Tyr Arg Asn Phe Asn Gly Asp Asp Lys Leu Gln Glu Cys Gly His Tyr  
                     100                                    105                                    110  
 Leu Phe Ser Glu Gly Ile Thr Ser Gly Cys Trp Phe Gly Lys Lys Glu  
                     115                                    120                                    125  
 Ile Arg Leu Tyr Glu Thr Phe Val Val Gln Leu Gln Asp Pro Arg Glu  
                     130                                    135                                    140  
 His Arg Lys Gln Pro Lys Gln Met Leu Lys Leu Gln Asp Leu Val Ile  
                     145                                    150                                    155                                    160  
 Pro Trp Ala Pro Glu Asn Leu Thr Leu Arg Asn Leu Ser Glu Phe Gln  
                     165                                    170                                    175  
 Leu Glu Leu Ser Trp Ser Asn Arg Tyr Leu Asp His Cys Leu Glu His  
                     180                                    185                                    190  
 Leu Val Gln Tyr Arg Ser Asp Arg Asp Arg Ser Trp Thr Glu Gln Ser  
                     195                                    200                                    205  
 Val Asp His Arg His Ser Phe Ser Leu Pro Ser Val Asp Ala Gln Lys  
                     210                                    215                                    220  
 Leu Tyr Thr Phe Arg Val Arg Ser Arg Tyr Asn Pro Leu Cys Gly Ser  
                     225                                    230                                    235                                    240  
 Ala Gln His Trp Ser Asp Trp Ser Tyr Pro Ile His Trp Gly Ser Asn  
                     245                                    250                                    255  
 Thr Ser Lys Glu Asn Ile Glu Asn Pro Glu Asn Pro Ser Leu Phe Ala  
                     260                                    265                                    270  
 Leu Glu Ala Val Leu Ile Pro Leu Gly Ser Met Gly Leu Ile Val Ser  
                     275                                    280                                    285  
 Leu Ile Cys Val Tyr Cys Trp Leu Glu Arg Thr Met Pro Arg Ile Pro  
                     290                                    295                                    300  
 Thr Leu Lys Asn Leu Glu Asp Leu Val Thr Glu Tyr Gln Gly Asn Phe  
                     305                                    310                                    315                                    320  
 Ser Ala Trp Ser Gly Val Ser Lys Gly Leu Ala Glu Ser Leu Gln Pro  
                     325                                    330                                    335  
 Asp Tyr Ser Glu Arg Leu Cys His Val Ser Glu Ile Pro Pro Lys Gly  
                     340                                    345                                    350  
 Gly Glu Gly Pro Gly Gly Ser Pro Cys Ser Gln His Ser Pro Tyr Trp  
                     355                                    360                                    365  
 Ala Pro Pro Cys Tyr Thr Leu Lys Pro Glu Pro  
                     370                                    375

<210> 4  
 <211> 733  
 <212> DNA  
 <213> Homo sapiens

<400> 4  
 gggatccgga gcccaaattct tctgacaaaa ctcacacatg cccaccgtgc ccagcacctg 60  
 aattcgaggg tgcaccgtca gtcttctctt tcccccaaa acccaaggac accctcatga 120  
 tctcccggac tcctgaggtc acatgcgtgg tggtagacgt aagccacgaa gaccctgagg 180  
 tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca aagccgcggg 240  
 aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg caccaggact 300  
 ggctgaatgg caaggagtac aagtgcagg tctccaacaa agccctcca acccccatcg 360  
 agaaaacccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac accctgcccc 420  
 catccccgga tgagctgacc aagaaccagg tcagcctgac ctgcctgggc aaaggcttct 480  
 atccaagcga catcgccgtg gagtgggaga gcaatgggca gccggagaac aactacaaga 540  
 ccacgcctcc cgtgctggac tccgacggct ccttcttct ctacagcaag ctccaccgtg 600  
 acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgcat gaggtctctg 660  
 acaaccacta cacgcagaag agcctctccc tgtctccggg taaatgagtg cgacggccgc 720  
 gactctagag gat 733

<210> 5  
 <211> 5  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (3)  
 <223> Xaa equals any amino acid

<400> 5  
 Trp Ser Xaa Trp Ser  
 1 5

<210> 6  
 <211> 86  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <221> Primer\_Bind  
 <223> Synthetic sequence with 4 tandem copies of the GAS binding site found in the IRF1 promoter (Rothman et al., Immunity 1:457-468 (1994)), 18 nucleotides complementary to the SV40 early promoter, and a Xho I restriction site.

<400> 6  
 ggcctcgag atttccccga aatctagatt tccccgaaat gatttcccgc aaatgatttc 60  
 cccgaaatat ctgcatctc aattag 86

<210> 7  
 <211> 27  
 <212> DNA  
 <213> Artificial Sequence

<220>



<221> Primer\_Bind

<223> Synthetic sequence complementary to the SV40 promoter; includes a Hind III restriction site.

<400> 7

gcggcaagct ttttgcaaag cctaggc

27

<210> 8

<211> 271

<212> DNA

<213> Artificial Sequence

<220>

<221> Protein\_Bind

<223> Synthetic promoter for use in biological assays; includes GAS binding sites found in the IRF1 promoter (Rothman et al., Immunity 1:457-468 (1994)).

<400> 8

ctcgagattt ccccgaaatc tagatttccc cgaaatgatt tccccgaaat gatttccccg 60  
aaatatctgc catctcaatt agtcagcaac catagtcccc cccctaactc cgcccatccc 120  
gcccctaact ccgcccagtt ccgcccattc tccgcccatt ggctgactaa ttttttttat 180  
ttatgcagag gccgaggccg cctcggcctc tgagctattc cagaagtagt gaggaggctt 240  
ttttggaggc ctaggctttt gcaaaaagct t 271

<210> 9

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<221> Primer\_Bind

<223> Synthetic primer complementary to human genomic EGR-1 promoter sequence (Sakamoto et al., Oncogene 6:867-871 (1991)); including an Xho I restriction site.

<400> 9

gcgctcgagg gatgacagcg atagaacccc gg

32

<210> 10

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<221> Primer\_Bind

<223> Synthetic primer complementary to human genomic EGR-1 promoter sequence (Sakamoto et al., Oncogene 6:867-871 (1991)); including an Hind III restriction site.

<400> 10

gcgaagcttc gcgactcccc ggatccgcct c

31

<210> 11

<211> 12

<212> DNA

<213> Homo sapiens

<400> 11

ggggactttc cc

12

<210> 12

<211> 73

<212> DNA

<213> Artificial Sequence

<220>

<221> Primer\_Bind

<223> Synthetic primer with 4 tandem copies of the NF-KB binding site (GGGGACTTTCCC), 18 nucleotides complementary to the 5' end of the SV40 early promoter sequence, and a XhoI restriction site.

<400> 12

ggggcctcga ggggactttc ccggggactt tccggggact ttccgggact ttccatcctg 60  
ccatctcaat tag 73

<210> 13

<211> 256

<212> DNA

<213> Artificial Sequence

<220>

<221> Protein\_Bind

<223> Synthetic promoter for use in biological assays, including NF-KB binding sites.

<400> 13

ctcgagggga ctttcccgga gactttccgg ggactttccg ggactttcca tctgccatct 60  
caattagtca gcaaccatag tcccgcctct aactccgccc atcccgcctc taactccgcc 120  
cagttccgcc cattctccgc cccatggctg actaattttt tttatttatg cagaggccga 180  
ggccgcctcg gcctctgagc tattccagaa gtagtgagga ggcttttttg gaggcctagg 240  
cttttgcaaa aagctt 256

<210> 14

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<221> Primer\_Bind

<223> Synthetic primer containing the Nco I restriction site followed a number of nucleotides of the amino terminal coding sequence of CRGCL

<400> 14

gtaggccat gggaggagca gcagaagga

29

<210> 15

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<221> Primer\_Bind

<223> Synthetic primer containing the BglII restriction site followed by a number nucleotides complementary to the 3' end of the coding sequence of CRCGCL

<400> 15

ggttaaagat ctcaacgcca cgtaggagcg gtc

33

<210> 16

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<221> Primer\_Bind

<223> Synthetic primer to amplify the cDNA sequence encoding the full length CRCGCL protein including the AUG initiation codon and the naturally associated leader, also contains a BglII site, and Kozak signal

<400> 16

ccggttagat ctgccatcat ggctttgggg caaggagg

38

<210> 17

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<221> Primer\_Bind

<223> Synthetic primer containing the XbaI restriction site followed by a number of nucleotides complementary to the 3' noncoding sequence of SEQ ID NO:1

<400> 17

ccggtttctga gatcacaagg ccacgtagga gcggtc

36

<210> 18

<211> 7

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (1)

<223> Xaa equals Ser, Thr, Gly or Leu

<220>

<221> SITE

<222> (2)

<223> Xaa equals any amino acid

<220>

<221> SITE  
<222> (4)  
<223> Xaa equals Ser or Gly

<220>  
<221> SITE  
<222> (5)  
<223> Xaa equals any amino acid

<400> 18  
Xaa Xaa Trp Xaa Xaa Trp Ser  
1 5

<210> 19  
<211> 7  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (2)  
<223> Xaa equals any amino acid

<220>  
<221> SITE  
<222> (5)  
<223> Xaa equals any amino acid

<400> 19  
Thr Xaa Pro Ser Xaa Trp Ser  
1 5

<210> 20  
<211> 7  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (2)  
<223> Xaa equals Pro or Glu

<220>  
<221> SITE  
<222> (3)  
<223> Xaa equals any amino acid

<220>  
<221> SITE  
<222> (4)  
<223> Xaa equals Val or Ile

<220>  
<221> SITE  
<222> (6)  
<223> Xaa equals Asn, Ser or Asp

&lt;400&gt; 20

Trp Xaa Xaa Xaa Pro Xaa Pro  
1 5

&lt;210&gt; 21

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (3)

&lt;223&gt; Xaa equals any amino acid

&lt;400&gt; 21

Ile Pro Xaa Val Pro Asp Pro  
1 5

&lt;210&gt; 22

&lt;211&gt; 54

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 22

Gln Ile Gln Ile Ile Tyr Phe Asn Leu Glu Thr Val Gln Val Thr Trp  
1 5 10 15Asn Ala Ser Lys Tyr Ser Arg Thr Asn Leu Thr Phe His Tyr Arg Phe  
20 25 30Asn Gly Asp Glu Ala Tyr Asp Gln Cys Thr Asn Tyr Leu Leu Gln Glu  
35 40 45Gly His Thr Ser Gly Cys  
50

&lt;210&gt; 23

&lt;211&gt; 30

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 23

Arg Arg His Ser Leu Phe Leu His Gln Glu Trp Asp Ala Pro Arg Phe  
1 5 10 15His Arg Lys Ser Leu Asp Gly Leu Leu Pro Glu Thr Gln Phe  
20 25 30

&lt;210&gt; 24

&lt;211&gt; 81

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 24

Leu Leu Tyr Glu Val Gln Tyr Arg Ser Pro Phe Asp Thr Glu Trp Gln

1	5	10	15
Ser Lys Gln Glu Asn Thr Cys Asn Val Thr Ile Glu Gly Leu Asp Ala	20	25	30
Glu Lys Cys Tyr Ser Phe Trp Val Arg Val Lys Ala Met Glu Asp Val	35	40	45
Tyr Gly Pro Asp Thr Tyr Pro Ser Asp Trp Ser Glu Val Thr Cys Trp	50	55	60
Gln Arg Gly Glu Ile Arg Asp Ala Cys Ala Glu Thr Pro Thr Pro Pro	65	70	75
			80

Lys

<210> 25  
 <211> 181  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (68)  
 <223> Xaa equals any amino acid

<220>  
 <221> SITE  
 <222> (73)  
 <223> Xaa equals any amino acid

<220>  
 <221> SITE  
 <222> (88)  
 <223> Xaa equals any amino acid

<400> 25
Met Glu Asp Val Tyr Gly Pro Asp Thr Tyr Pro Ser Asp Trp Ser Glu
1 5 10 15
Val Thr Cys Trp Gln Arg Gly Glu Ile Arg Asp Ala Cys Ala Glu Thr
20 25 30
Pro Thr Pro Pro Lys Pro Lys Leu Ser Lys Phe Ile Leu Ile Ser Ser
35 40 45
Leu Ala Ile Leu Leu Met Val Ser Leu Leu Leu Leu Ser Leu Trp Lys
50 55 60
Leu Trp Arg Xaa Lys Lys Phe Leu Xaa Pro Ser Val Pro Asp Pro Lys
65 70 75 80
Ser Ile Phe Pro Gly Leu Phe Xaa Ile His Gln Gly Asn Phe Gln Glu
85 90 95
Trp Ile Thr Asp Thr Gln Asn Val Ala His Leu His Lys Met Ala Gly
100 105 110

Ala Glu Gln Glu Ser Gly Pro Glu Glu Pro Leu Val Val Gln Leu Ala  
 115 120 125

Lys Thr Glu Ala Glu Ser Pro Arg Met Leu Asp Pro Gln Thr Glu Glu  
 130 135 140

Lys Glu Ala Ser Gly Gly Ser Leu Gln Leu Pro His Gln Pro Leu Gln  
 145 150 155 160

Gly Gly Asp Val Val Thr Ile Gly Gly Phe Thr Phe Val Met Asn Asp  
 165 170 175

Arg Ser Tyr Val Ala  
 180

<210> 26

<211> 1567

<212> DNA

<213> Homo sapiens

<220>

<221> misc\_feature

<222> (830)

<223> n equals a, t, g or c

<220>

<221> misc\_feature

<222> (416)

<223> y equals c or t

<220>

<221> misc\_feature

<222> (784)

<223> m equals a or c

<220>

<221> misc\_feature

<222> (785)

<223> y equals c or t

<400> 26

gggcatgggg cggctgggtc tgctgtgggg agctgccgtc tttctgctgg gaggtggat 60  
 ggctttgggg caaggaggag cagcagaagg agtacagatt caratcatct acttcaattt 120  
 agaaaccgtg caggtgacat ggaatgccag caaatactcc aggaccaacc tgactttcca 180  
 ctacagattc aacgggtgat aggcctatga ccagtgcacc aactaccttc tccaggaagg 240  
 tcacacttcg gggcgctcc tagacgasa gcagcgagac gacattctct atttctccat 300  
 caggaatggg acgcaccccg ttttcaccgc aagtcgctgg atgggtttatt acctgaaacc 360  
 cagttccccg aagcacgtga gatttcgtgg catcaggaaw gacggtgacg tggtcycgac 420  
 ctgtcctacg gggatctcct ctatgaggtt cagtaccgga gccccttcga caccgagtgg 480  
 cagtccaaac aggaaaatac ctgcaacgtc accatagaag gcttggatgc cgagaagtg 540  
 tactctttct gggtcagggt gaaggctatg gaggatgtat atgggccaga cacataccca 600  
 agcgactggg cagaggtgac atgctggcag agaggcgaga ttcgggatgc ctgtgcagag 660  
 acaccaacgc ctcccaaacc aaagctgtcc aaatttattt taatttccag cctggccatc 720  
 cttctgatgg tgtctctcct cttctgtct ttatggaaat tatggagat gaagaagttt 780  
 ctcmytccca gcgtgccaga cccgaaatcc atcttccccg ggctctttgn tatacaccaa 840  
 gggaaacttc aggagtggat cacagacacc cagaacgtgg ccacactcca caagatggca 900  
 ggtgcagagc aagaaagtgg ccccgaggag cccttggtag tccagttggc caagactgaa 960

```

gccgagtctc ccaggatgct ggacccacag accgaggaga aagaggcctc tgggggatcc 1020
ctccagcttc cccaccagcc cctccaaggc ggtgatgtgg tcacaatcgg gggcttcacc 1080
tttgtgatga atgaccgctc ctacgtggcg ttgtgatgga cacaccactg tcaaagtcaa 1140
cgtcaggatc cacgttgaca tttaaagaca gaggggactg tcccggggac tccacaccac 1200
catggatggg aagtctccac gccaatgatg gtaggactag gagactctga agaccagcc 1260
tcaccgccta atgcggccac tgccctgcta actttccccc acatgagtct ctgtgttcaa 1320
aggcttgatg gcagatggga gccaatgtct ccaggagatt tactcccagt tccttttcgt 1380
gctgaacggt gtcacataaa cccaaggca gcacgtccaa aatgctgtaa aaccatcttc 1440
ccactctgtg agtccccagt tccgtccatg taccattccc atagcattgg attctcggag 1500
gattttttgt ctgttttgag actccaaacc acctctaccc ctacaaaaaa aaaaaaaaaa 1560
aactcga                                     1567

```

<210> 27  
 <211> 170  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (89)  
 <223> Xaa equals any amino acid

<220>  
 <221> SITE  
 <222> (132)  
 <223> Xaa equals any amino acid

<220>  
 <221> SITE  
 <222> (138)  
 <223> Xaa equals any amino acid

<400> 27  
 Met Gly Arg Leu Val Leu Leu Trp Gly Ala Ala Val Phe Leu Leu Gly  
 1 5 10 15  
 Gly Trp Met Ala Leu Gly Gln Gly Gly Ala Ala Glu Gly Val Gln Ile  
 20 25 30  
 Gln Ile Ile Tyr Phe Asn Leu Glu Thr Val Gln Val Thr Trp Asn Ala  
 35 40 45  
 Ser Lys Tyr Ser Arg Thr Asn Leu Thr Phe His Tyr Arg Phe Asn Gly  
 50 55 60  
 Asp Glu Ala Tyr Asp Gln Cys Thr Asn Tyr Leu Leu Gln Glu Gly His  
 65 70 75 80  
 Thr Ser Gly Cys Leu Leu Asp Ala Xaa Gln Arg Asp Asp Ile Leu Tyr  
 85 90 95  
 Phe Ser Ile Arg Asn Gly Thr His Pro Val Phe Thr Ala Ser Arg Trp  
 100 105 110  
 Met Val Tyr Tyr Leu Lys Pro Ser Ser Pro Lys His Val Arg Phe Arg  
 115 120 125  
 Gly Ile Arg Xaa Asp Gly Asp Val Phe Xaa Thr Cys Pro Thr Gly Ile



130	135	140
Ser Ser Met Arg Phe Ser Thr Gly Ala Pro Ser Thr Pro Ser Gly Ser		
145	150	155 160
Pro Asn Arg Lys Ile Pro Ala Thr Ser Pro		
165	170	

<210> 28  
 <211> 36  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <221> Primer\_Bind  
 <223> Synthetic primer to amplify the cDNA sequence encoding the amino acids Met 1 to Lys 231 including the AUG initiation codon and the naturally associated leader,also contains a BglII site,and Kozak signal

<400> 28  
 ccggtagat ctgccatcat ggggcggctg gttctg 36

<210> 29  
 <211> 31  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <221> Primer\_Bind  
 <223> Synthetic primer to amplify the cDNA sequence encoding the amino acids Met 1 to Lys 231,also contains a BglII site

<400> 29  
 ggccggtcta gatttgga gctttggtt g 31

<210> 30  
 <211> 4  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (2)  
 <223> Xaa equals any amino acid

<400> 30  
 Trp Xaa Trp Ser  
 1

<210> 31  
 <211> 34  
 <212> DNA  
 <213> Artificial Sequence

<220>

<221> Primer\_Bind

<223> Synthetic primer to amplify the cDNA sequence encoding the amino acids Met 1 to Lys 231, also contains a XbaI site

<400> 31

ggccggtcta gattatttgg acagctttgg ttg

34

<210> 32

<211> 144

<212> DNA

<213> Artificial Sequence

<220>

<221> Primer\_Bind

<223> Synthetic primer to amplify DNA encoding amino acids M1-K231 of SEQ ID NO:2, contains a BglII restriction site.

<400> 32..

cgggttagat ctgccatcat ggggcggctg gttctgccgg ttagatctgc catcatgggg 60  
cggctgggtc tgccggttag atctgccatc atggggcggc tggttctgcc ggtagatct 120  
gccatcatgg ggcggtggt tctg 144